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	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR		
☐	L9	L8 and (CDT)	3
☐	L8	L7 and deficient	43
☐	L7	L6 and lacking	103
☐	L6	L5 and carbohydrate	191
☐	L5	anti-transferrin	442
☐	L4	(althaus)adj(harald)	21
☐	L3	(antibod?)same(carbohydrate)adj(deficient)adj(transferrin)	8
	DB=DWPI; PLUR=YES; OP=OR		
☐	L2	0605627	3
☐	L1	EP 0605627	1601642

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=> file medline embase biosis scisearch caplus
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 13:39:01 ON 06 NOV 2005
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FILE 'EMBASE' ENTERED AT 13:39:01 ON 06 NOV 2005

FILE 'BIOSIS' ENTERED AT 13:39:01 ON 06 NOV 2005

FILE 'SCISEARCH' ENTERED AT 13:39:01 ON 06 NOV 2005

FILE 'CAPLUS' ENTERED AT 13:39:01 ON 06 NOV 2005

=> s carbohydrate deficient transferrin antibod?
L1 2 CARBOHYDRATE DEFICIENT TRANSFERRIN ANTIBOD?

=> dup remove l1
PROCESSING COMPLETED FOR L1
L2 2 DUP REMOVE L1 (0 DUPLICATES REMOVED)

=> d l2 1-2 cbib abs

L2 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN
2004:17426 Document No. 140:56037 Anti-carbohydrate deficient transferrin (CDT) specific antibody and method for its production. Althaus, Harald (Dade Behring Marburg G.m.b.H., Germany). Eur. Pat. Appl. EP 1378521 A1 20040107, 21 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK. (German). CODEN: EPXXDW. APPLICATION: EP 2003-11334 20030519. PRIORITY: DE 2002-10230550 20020705.
AB The invention concerns antibodies to carbohydrate deficient transferrin (CDT) that bind to the following sequences in CDT: (1) WARSMGGKEDLIWELL ; (2) TTEDSIAKIMNGEADAMSLDGGF; (3) SKLSMGSGLNLSEPN; (4) YEKYLGEEYVKAV. The antibodies bind to CDT in aqueous solns. without a solid phase. For the production of monoclonal antibodies animals are immunized with non-glycosylated transferrin; spleen cells of the animals are fused with myeloma cells, thus antibody-producing hybrid cells are produced. The antibodies can be used for serodiagnosis of alc. patients.

L2 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2004:369789 Document No.: PREV200400367719. Alcohol misuse increases serum antibodies to oxidized LDL and C-reactive protein. Alho, Hannu; Sillanaukee, Pekka; Kalela, Anne; Jaakkola, Olli; Laine, Seppo; Nikkari, Seppo T. [Reprint Author]. Sch MedDept Med Biochem, Univ Tampere, FIN-33014, Tampere, Finland. seppo.nikkari@uta.fi. Alcohol and Alcoholism, (July 2004) Vol. 39, No. 4, pp. 312-315. print.
CODEN: ALALDD. ISSN: 0735-0414. Language: English.
AB Aims: To clarify the relationship of alcohol consumption with serum antibodies to oxidized low-density lipoprotein (oxLDL) and the inflammation marker C-reactive protein (CRP). Methods: The study population consisted of 280 men with evidence of alcohol misuse by having self-reported alcohol consumption values over 280 g absolute ethanol per week and 250 age-matched moderate drinkers from a population of Finnish men participating in the FINRISK survey study. Serum samples were analysed for antibodies to oxLDL, C-reactive protein (CRP), total cholesterol, HDL-cholesterol, triglycerides, carbohydrate-deficient transferrin (CDT) and gamma-glutamyl transferase (GGT). The characteristics of the top and bottom half of the alcohol misusers, in regard to weekly alcohol consumption, were compared with the controls. Results: Serum antibody titres to oxLDL were higher in the top half and the levels of CRP, HDL-cholesterol, triglycerides, GGT and CDT were elevated in both the top half and the bottom half of the alcohol misusers, compared to controls. Conclusion: We propose that alcohol misuse may

result in increased inflammation leading to oxidation of LDL.

=> s carbohydrate deficient transferrin
L3 2673 CARBOHYDRATE DEFICIENT TRANSFERRIN

=> s l3 and antibod?
L4 148 L3 AND ANTIBOD?

=> s l4 and VVARSMGGKEDLIWELL
L5 0 L4 AND VVARSMGGKEDLIWELL

=> s l4 and TTEDSIAKIMNGEADAMSLDGGF
L6 1 L4 AND TTEDSIAKIMNGEADAMSLDGGF

=> d l6 cbib abs

L6 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2005 ACS on STN
2004:17426 Document No. 140:56037 Anti-**carbohydrate deficient transferrin** (CDT) specific **antibody** and method for its production. Althaus, Harald (Dade Behring Marburg G.m.b.H., Germany). Eur. Pat. Appl. EP 1378521 A1 20040107, 21 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK. (German). CODEN: EPXXDW. APPLICATION: EP 2003-11334 20030519. PRIORITY: DE 2002-10230550 20020705.
AB The invention concerns **antibodies** to **carbohydrate deficient transferrin** (CDT) that bind to the following sequences in CDT: (1) WARSMGGKEDLIWELL ; (2) **TTEDSIAKIMNGEADAMSLDGGF**; (3) SKLSMGSGLNLSEPN; (4) YEKYLGEEYVKAV. The **antibodies** bind to CDT in aqueous solns. without a solid phase. For the production of monoclonal **antibodies** animals are immunized with non-glycosylated transferrin; spleen cells of the animals are fused with myeloma cells, thus **antibody**-producing hybrid cells are produced. The **antibodies** can be used for serodiagnosis of alc. patients.

=> s l4 and SKLSMGSGLNLSEPN
L7 1 L4 AND SKLSMGSGLNLSEPN

=> d l7 cbib abs

L7 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2005 ACS on STN
2004:17426 Document No. 140:56037 Anti-**carbohydrate deficient transferrin** (CDT) specific **antibody** and method for its production. Althaus, Harald (Dade Behring Marburg G.m.b.H., Germany). Eur. Pat. Appl. EP 1378521 A1 20040107, 21 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK. (German). CODEN: EPXXDW. APPLICATION: EP 2003-11334 20030519. PRIORITY: DE 2002-10230550 20020705.
AB The invention concerns **antibodies** to **carbohydrate deficient transferrin** (CDT) that bind to the following sequences in CDT: (1) WARSMGGKEDLIWELL ; (2) TTEDSIAKIMNGEADAMSLDGGF; (3) **SKLSMGSGLNLSEPN**; (4) YEKYLGEEYVKAV. The **antibodies** bind to CDT in aqueous solns. without a solid phase. For the production of monoclonal **antibodies** animals are immunized with non-glycosylated transferrin; spleen cells of the animals are fused with myeloma cells, thus **antibody**-producing hybrid cells are produced. The **antibodies** can be used for serodiagnosis of alc. patients.

=> s l4 and YEKYLGEEYVKAV

```
L8            1 L4 AND YEKYLGEEYVKAV

=> d l8 cbib abs

L8   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2005 ACS on STN
2004:17426   Document No. 140:56037  Anti-carbohydrate
     deficient transferrin (CDT) specific antibody
     and method for its production.  Althaus, Harald (Dade Behring Marburg
     G.m.b.H., Germany).  Eur. Pat. Appl. EP 1378521 A1 20040107, 21 pp.
     DESIGNATED STATES: R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL,
     SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK.
     (German).  CODEN: EPXXDW.  APPLICATION: EP 2003-11334 20030519.  PRIORITY:
     DE 2002-10230550 20020705.
AB   The invention concerns antibodies to carbohydrate
     deficient transferrin (CDT) that bind to the following
     sequences in CDT: (1) WARSMGGKEDLIWELL ; (2) TTEDSIAKIMNGEADAMSLDGGF; (3)
     SKLSMGSGLNLSEPN; (4) YEKYLGEEYVKAV.  The antibodies
     bind to CDT in aqueous solns. without a solid phase.  For the production of
     monoclonal antibodies animals are immunized with
     non-glycosylated transferrin; spleen cells of the animals are fused with
     myeloma cells, thus antibody-producing hybrid cells are
     produced.  The antibodies can be used for serodiagnosis of alc.
     patients.


=> s l4 and DSM ACC2540
L9            0 L4 AND DSM ACC2540

=> s "DSM ACC2540"
L10           0 "DSM ACC2540"

=> s "DSM ACC2541"
L11           0 "DSM ACC2541"

=> s ACC2541"
MISMATCHED QUOTE 'ACC2541"'
Quotation marks (or apostrophes) must be used in pairs,
one before and one after the expression you are setting
off or masking.

=> s "ACC2541"
L12           0 "ACC2541"

=> s "ACC 2540"
L13           0 "ACC 2540"

=> d his

     (FILE 'HOME' ENTERED AT 13:38:49 ON 06 NOV 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 13:39:01 ON
     06 NOV 2005
L1             2 S CARBOHYDRATE DEFICIENT TRANSFERRIN ANTIBOD?
L2             2 DUP REMOVE L1 (0 DUPLICATES REMOVED)
L3          2673 S CARBOHYDRATE DEFICIENT TRANSFERRIN
L4           148 S L3 AND ANTIBOD?
L5             0 S L4 AND VVARSMGGKEDLIWELL
L6             1 S L4 AND TTEDSIAKIMNGEADAMSLDGGF
L7             1 S L4 AND SKLSMGSGLNLSEPN
L8             1 S L4 AND YEKYLGEEYVKAV
L9             0 S L4 AND DSM ACC2540
L10            0 S "DSM ACC2540"
L11            0 S "DSM ACC2541"
L12            0 S "ACC2541"
L13            0 S "ACC 2540"
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=> dup remove l4
PROCESSING COMPLETED FOR L4
L14 57 DUP REMOVE L4 (91 DUPLICATES REMOVED)

=> d l14 1-57 cbib abs

L14 ANSWER 1 OF 57 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN
2005240834 EMBASE **Carbohydrate-deficient transferrin** measured by capillary zone electrophoresis and by turbidimetric immunoassay for identification of young heavy drinkers. Daeppen J.-B.; Anex F.; Favrat B.; Bissery A.; Leutwyler J.; Gammeter R.; Mangin P.; Augsburger M.. J.-B. Daeppen, Alcohol Treatment Center, CHUV, Mont-Paisible 16, 1011 Lausanne, Switzerland. jean-bernard.daeppen@inst.hospvd.ch. Clinical Chemistry Vol. 51, No. 6, pp. 1046-1048 2005.
Refs: 13.
ISSN: 0009-9147. CODEN: CLCHAU
Pub. Country: United States. Language: English.
ED Entered STN: 20050630
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L14 ANSWER 2 OF 57 CAPLUS COPYRIGHT 2005 ACS on STN
2004:17426 Document No. 140:56037 Anti-**carbohydrate deficient transferrin** (CDT) specific **antibody** and method for its production. Althaus, Harald (Dade Behring Marburg G.m.b.H., Germany). Eur. Pat. Appl. EP 1378521 A1 20040107, 21 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK. (German). CODEN: EPXXDW. APPLICATION: EP 2003-11334 20030519. PRIORITY: DE 2002-10230550 20020705.
AB The invention concerns **antibodies** to **carbohydrate deficient transferrin** (CDT) that bind to the following sequences in CDT: (1) WARSMGGKEDLIWELL ; (2) TTEDSIAKIMNGEADAMSLDGGF; (3) SKLSMGSGLNLSEPN; (4) YEKYLGEEYVKAV. The **antibodies** bind to CDT in aqueous solns. without a solid phase. For the production of monoclonal **antibodies** animals are immunized with non-glycosylated transferrin; spleen cells of the animals are fused with myeloma cells, thus **antibody**-producing hybrid cells are produced. The **antibodies** can be used for serodiagnosis of alc. patients.

L14 ANSWER 3 OF 57 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2004:271234 The Genuine Article (R) Number: 803IQ. High-throughput comprehensive analysis of human plasma proteins: A step toward population proteomics. Nedelkov D (Reprint); Tubbs K A; Niederkofler E E; Kiernan U A; Nelson R W. Instrins Bioprobes Inc, 625 S Smith Rd, Suite 22, Tempe, AZ 85281 USA (Reprint); Instrins Bioprobes Inc, Tempe, AZ 85281 USA. ANALYTICAL CHEMISTRY (15 MAR 2004) Vol. 76, No. 6, pp. 1733-1737. ISSN: 0003-2700. Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB A high-throughput (HT) comprehensive analysis approach was developed for assaying proteins directly from human plasma. Proteins were selectively retrieved, by utilizing **antibodies** immobilized within affinity pipet tips, and eluted onto enzymatically active mass spectrometer targets for subsequent digestion and structural characterization. Several parameters, including uniform parallel protein elution from 96 affinity pipet tips, proper buffering for on-target digestion, termination of the digestion, and MALDI matrix (re)introduction, were evaluated and optimized. The approach was validated via parallel, high-throughput analysis of transthyretin (M) and transferrin (TRFE) from 96 identical plasma samples. The 96 parallel analyses for each protein were completed in less than 90 min, measured

from protein extraction to insertion in the mass spectrometer. Virtually identical mass spectra were obtained from the 96 TTR analyses, characterized by the presence of 14 tryptic fragments that allowed TTR sequence mapping with 100% coverage. Database search returned TTR as the best match for all 96 data sets. In regard to the TRFE analyses, database searching using data from the 96 spectra returned TRFE as the best match for all but 1 of the spectra. TRFE was mapped with 47-69% sequence coverage, with gaps in the sequence coverage corresponding to the carbohydrate-containing peptide fragments and large and small trypsin fragments that fell outside the window of mass analysis. Overall, the combined high-throughput affinity capture-protein digestion approach showed high reproducibility and speed and yielded an exceptional level of protein characterization, suggesting its use in future population proteomics endeavors.

L14 ANSWER 4 OF 57 MEDLINE on STN DUPLICATE 1

2004489101. PubMed ID: 15365311. CDT values are not influenced by epithelial cell apoptosis in chronic alcoholic patients-preliminary results. Ramskogler Katrin; Brunner Markus; Hertling Ines; Dvorak Alexander; Kapusta Nestor; Krenn Claus; Moser Bernhard; Roth Georg; Lesch Otto Michael; Ankersmit Hendrik Jan; Walter Henriette. (Department of Psychiatry, Medical University of Vienna, Vienna, Austria.) Alcoholism, clinical and experimental research, (2004 Sep) 28 (9) 1396-8. Journal code: 7707242. ISSN: 0145-6008. Pub. country: United States. Language: English.

AB **Carbohydrate-deficient transferrin** (CDT) has been well established as a marker for high alcohol consumption. As studies concerning the specificity of CDT in patients with liver disease have shown controversial outcomes, efforts to illuminate mechanisms leading to impaired CDT specificity in this patient group cannot yet be considered successful. Evidence of apoptosis as examined in 72 alcohol-dependent patients using serum contents of caspase-related M30 monoclonal **antibody** significantly correlated with aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transpeptidase but did not influence CDT levels. These results suggest that impairment of CDT specificity is brought forth by derangement of hepatic metabolism rather than by acute hepatocellular damage.

L14 ANSWER 5 OF 57 MEDLINE on STN DUPLICATE 2

2004355898. PubMed ID: 15208162. Alcohol misuse increases serum **antibodies** to oxidized LDL and C-reactive protein. Alho Hannu; Sillanaukee Pekka; Kalela Anne; Jaakkola Olli; Laine Seppo; Nikkari Seppo T. (Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland.) Alcohol and alcoholism (Oxford, Oxfordshire), (2004 Jul-Aug) 39 (4) 312-5. Journal code: 8310684. ISSN: 0735-0414. Pub. country: England: United Kingdom. Language: English.

AB AIMS: To clarify the relationship of alcohol consumption with serum **antibodies** to oxidized low-density lipoprotein (oxLDL) and the inflammation marker C-reactive protein (CRP). METHODS: The study population consisted of 280 men with evidence of alcohol misuse by having self-reported alcohol consumption values over 280 g absolute ethanol per week and 250 age-matched moderate drinkers from a population of Finnish men participating in the FINRISK survey study. Serum samples were analysed for **antibodies** to oxLDL, C-reactive protein (CRP), total cholesterol, HDL-cholesterol, triglycerides, **carbohydrate-deficient transferrin** (CDT) and gamma-glutamyl transferase (GGT). The characteristics of the top and bottom half of the alcohol misusers, in regard to weekly alcohol consumption, were compared with the controls. RESULTS: Serum **antibody** titres to oxLDL were higher in the top half and the levels of CRP, HDL-cholesterol, triglycerides, GGT and CDT were elevated in both the top half and the bottom half of the alcohol misusers, compared to controls. CONCLUSION: We propose that alcohol misuse may result in increased inflammation leading to oxidation of LDL.

L14 ANSWER 6 OF 57 CAPLUS COPYRIGHT 2005 ACS on STN
2004:461459 Document No. 141:238121 **Carbohydrate-deficient transferrin** (CDT) as a biomarker in persons suspected of alcohol abuse. Golka, Klaus; Sondermann, Rolf; Reich, Susanne E.; Wiese, Andreas (Institut fur Arbeitsphysiologie, Universitat Dortmund (IfADo), Dortmund, D-44139, Germany). Toxicology Letters, 151(1), 235-241 (English) 2004. CODEN: TOLED5. ISSN: 0378-4274. Publisher: Elsevier Science Ireland Ltd..

AB The coherence of **carbohydrate-deficient transferrin** (CDT) as a biomarker of alc. abuse was investigated with 15 conventional laboratory parameters, with the self-reported medical history and with clin. findings, all previously reported to be associated with chronic alc. intake. In total, 100 male persons who were at least suspected of abusing alc. were assessed. Medical history, clin. picture, and phys. examination were taken, and laboratory parameters regarding blood count, liver enzymes, serum lipids, iron balance, IgA, and uric acid were determined These data were correlated with the CDT values, the daily ethanol intakes reported, and several findings from medical history and clin. examination The mean CDT level (mean±S.D.) of the entire group was 29.4±19.7 U/L. Eighty-one patients admitted a daily ethanol intake of 60 g or more. The ratio AST/ALT (de Ritis ratio) appeared as the best conventional parameter correlated with both CDT and ethanol intake. Mean corpuscular volume (MCV), serum iron, AST, and red blood cell count also correlated significantly with CDT. CDT, AST, and ferritin correlated significantly with the reported daily ethanol intake. Apparently, CDT provides a reliable estimate of long-term alc. intake.

L14 ANSWER 7 OF 57 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2004:829384 The Genuine Article (R) Number: 851GO. Diagnosis and therapy of alcoholic liver disease. Levitsky J; Mailliard M E (Reprint). Univ Nebraska, Coll Med, Div Gastroenterol & Hepatol, Box 982000, Omaha, NE 68583 USA (Reprint); Univ Nebraska, Coll Med, Div Gastroenterol & Hepatol, Omaha, NE 68583 USA; Univ Nebraska, Ctr Med, Dept Internal Med, Coll Med, Omaha, NE 68583 USA; Vet Affairs Med Ctr, Omaha, NE USA. mmaillia@unmc.edu . SEMINARS IN LIVER DISEASE (AUG 2004) Vol. 24, No. 3, pp. 233-247. ISSN: 0272-8087. Publisher: THIEME MEDICAL PUBL INC, 333 SEVENTH AVE, NEW YORK, NY 10001 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Alcoholic liver disease (ALD) presents considerable challenges to clinicians. Screening for alcohol abuse and alcoholism should be routine and repeated annually with close attention to signs and symptoms of liver disease. In patients with evidence of liver dysfunction or injury, consideration should be given to performance of liver biopsy for diagnosis and prognosis and prior to initiation of medication with the potential for significant side effects. Therapy depends on the spectrum of pathological liver injury: alcoholic fatty liver, alcoholic hepatitis, and cirrhosis. Abstention is the foundation of therapy for an alcohol problem. Alcoholic fatty liver should improve with abstention, but the similarity to the pathogenesis of nonalcoholic fatty liver and potential for progressive injury merits consideration of lipotropic agents. The continuing mortality, poor acceptance of corticosteroids, and identification of tumor necrosis factor-alpha (TNF-alpha) as an integral component has led to studies of pentoxifylline and, recently, anti-TNF **antibody** to neutralize cytokines in the therapy of severe alcoholic hepatitis. Antioxidant therapy of alcoholic cirrhosis has significant promise but will require large clinical trials.

L14 ANSWER 8 OF 57 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2004:459742 The Genuine Article (R) Number: 820BG. 5-and 6-glycosylation of transferrin in patients with Alzheimer's disease. van Rensburg S J (Reprint); Berman P; Potocnik F; MacGregor P; Hon D; de Villiers N. Tygerberg Hosp, Dept Chem Pathol, Natl Hlth Lab Serv, POB 19113, ZA-7505

Tygerberg, South Africa (Reprint); Tygerberg Hosp, Dept Chem Pathol, Natl Hlth Lab Serv, ZA-7505 Tygerberg, South Africa; Univ Stellenbosch, ZA-7600 Stellenbosch, South Africa; Groote Schuur Hosp, Dept Chem Pathol, Natl Hlth Lab Serv, Cape Town, South Africa; Univ Cape Town, ZA-7700 Rondebosch, South Africa; Tygerberg Hosp, Dept Psychiat, ZA-7505 Tygerberg, South Africa; Tygerberg Hosp, Informat Management Unit, ZA-7505 Tygerberg, South Africa; Genecare Mol Genet Pty Ltd, Cape Town, South Africa. METABOLIC BRAIN DISEASE (JUN 2004) Vol. 19, No. 1-2, pp. 89-96. ISSN: 0885-7490. Publisher: KLUWER ACADEMIC/PLENUM PUBL, 233 SPRING ST, NEW YORK, NY 10013 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Transferrin is a glycosylated metal-carrying serum protein. One of the biological functions of glycosylation is to regulate the life span of proteins, less glycosylation leading to a faster clearance of a protein from the circulation. In the case of transferrin, this would indirectly also influence iron homeostasis. Higher glycosylation has been demonstrated in patients with Parkinson's disease and rheumatoid arthritis. A genetic variant of transferrin, TfC2, occurs with increased frequency in patients with Alzheimer's disease (AD), rheumatoid arthritis, and other diseases associated with a free radical etiology. Investigations have so far not revealed the reason for the pro-oxidative qualities of TfC2. In this study the glycosylation of Tf in AD (TfC1 homozygotes and TfC1C2 heterozygotes) was compared with alcohol-induced dementia (AID) patients and nondemented, age-matched controls, using isoelectric focusing followed by blotting with anti-Tf **antibodies** . In TfC1 homozygotes a shift was found toward higher sialylation, but in TfC1C2 heterozygotes the 5- and 6-sialylated bands were less concentrated. The decreased sialalytion found for TfC1C2 heterozygotes, may indicate that the pro-oxidative TfC2 molecules are removed from the circulation at a faster rate than TfC1. This may be of benefit to AD patients having TfC2, but still does not explain why this Tf variant is pro-oxidative.

L14 ANSWER 9 OF 57 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2005:320560 Document No.: PREV200510106919. A novel particle-enhanced assay for the immuno-nephelometric determination of **carbohydrate-deficient transferrin.** Kraul, D. [Reprint Author]; Hackler, R.; Althaus, H.. Univ Marburg, Klin Innere Med Kardiol, AG Pravent Kardiol, D-35032 Marburg, Germany. Alcoholism Clinical and Experimental Research, (AUG 2004) Vol. 28, No. 8, Suppl. S, pp. 34A. Meeting Info.: 12th International Congress of the International-Society-for-Biomedical-Research-on-Alcoholism. Heidelberg, GERMANY. September 29 -October 02, 2004. Int Soc Biomed Res Alcoholism. CODEN: ACRSDM. ISSN: 0145-6008. Language: English.

L14 ANSWER 10 OF 57 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2005:320557 Document No.: PREV200510106916. Evaluation of Dade Behring N Latex CDT*: A novel homogeneous immunoassay for **carbohydrate-deficient transferrin.** Helander, A. [Reprint Author]; Dahl, H.; Swanson, I.; Bergstrom, J.. Karolinska Inst and Univ Hosp, SE-17176 Stockholm, Sweden. Alcoholism Clinical and Experimental Research, (AUG 2004) Vol. 28, No. 8, Suppl. S, pp. 33A. Meeting Info.: 12th International Congress of the International-Society-for-Biomedical-Research-on-Alcoholism. Heidelberg, GERMANY. September 29 -October 02, 2004. Int Soc Biomed Res Alcoholism. CODEN: ACRSDM. ISSN: 0145-6008. Language: English.

L14 ANSWER 11 OF 57 MEDLINE on STN DUPLICATE 3
2003517798. PubMed ID: 14578317. Analyte comigrating with trisialotransferrin during capillary zone electrophoresis of sera from patients with cancer. Ramdani Brahim; Nuyens Vincent; Codden Thierry; Perpete Gael; Colicis Jacques; Lenaerts Anne; Henry Jean-Pol; Legros Franz J. (University Department of Gastroenterology, Centre Hospitalier Universitaire de Charleroi, 92, Boulevard Janson, 6000 Charleroi, Belgium.) Clinical chemistry, (2003 Nov) 49 (11) 1854-64. Journal code: 9421549.

ISSN: 0009-9147. Pub. country: United States. Language: English.

AB BACKGROUND: Serum concentrations of monoglycosylated isoforms of transferrin are increased by chronic ethanol intake. We investigated transferrin glycosylation in patients with cancer, in which aberrant glycosylation is also induced. METHODS: We used a P/ACE 5000 series capillary zone electrophoresis (CZE) apparatus and a CZE **carbohydrate-deficient transferrin** reagent set to study 200 cancer patients who consumed alcohol moderately and 33 who were alcohol abusers; we then compared these patients with 56 healthy teetotalers, 89 moderate, and 112 excessive alcohol drinkers without known malignancies. Transferrin isoforms were identified by immunosubtraction with anti-human transferrin polyclonal **antibody.** RESULTS: Seven peaks, P0-P6, were visualized and completely or partly immunosubtracted when CZE separation was performed at pH 8.5. P0 was present in 95% of alcohol abusers with or without cancer. P3 was significantly higher in cancer patients and was only partly immunosubtracted as trisialotransferrin in all groups. The comigrating analyte was not altered by papain, precipitation by ethanol, or extraction by organic solvents, but was sensitive to acid hydrolysis, suggesting a polysaccharide structure. When isolated at pH 8.25, this analyte was higher in cancer patients. ROC curve analysis identified localized malignant neoplasia at P3 values above 5.8% of total transferrin (sensitivity, 0.78; specificity, 0.87), regardless of alcohol consumption. Disseminated cancers were better differentiated above 8% (sensitivity, 0.94; specificity, 0.96). CONCLUSIONS: Malignant neoplasia, unlike excessive ethanol intake, did not alter the addition of two N-glycans to transferrin. A peak comigrating with trisialotransferrin had characteristics of a polysaccharide in all adults and was increased in sera of patients with cancer.

L14 ANSWER 12 OF 57 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

2004019741 EMBASE Mass spectrometric analysis of human transferrin in different body fluids. Kleinert P.; Kuster T.; Durka S.; Ballhausen D.; Bosshard N.U.; Steinmann B.; Hanseler E.; Jaeken J.; Heizmann C.W.; Troxler H.. Dr. H. Troxler, Department of Pediatrics, Division of Clinical Chemistry, University of Zurich, Steinwiesstr. 75, 8032 Zurich, Switzerland. heinz.troxler@kispi.unizh.ch. Clinical Chemistry and Laboratory Medicine Vol. 41, No. 12, pp. 1580-1588 2003.
Refs: 27.
ISSN: 1434-6621. CODEN: CCLMFW
Pub. Country: Germany. Language: English. Summary Language: English.

ED Entered STN: 20040122

AB In this study, we present a versatile new procedure for the analysis of transferrin and its isoforms isolated from human body fluids such as serum, plasma, and cerebrospinal fluid. This method is based on a three-step procedure: (i) isolation of transferrins using anion-exchange chromatography with UV detection; (ii) concentration of the transferrin fraction; (iii) detection of the transferrins with liquid chromatography-electrospray mass spectrometry. Pre-analytical sample procedures can be omitted and no immunoaffinity columns or transferrin-specific immunoassays were used. Anticoagulants such as heparin, EDTA, citrate, and oxalate do not interfere with our analysis. According to their respective molecular masses, up to ten different isoforms of transferrin could be identified in a serum sample from a patient with a congenital disorder of glycosylation type Ia (CDG-Ia). The method was successfully applied to different pathological samples from patients with CDG-Ia, CDG-Ib, CDG-Ic, CDG-Ie, CDG-If, and CDG-IIa. Additionally, samples from alcohol consumers that were found with turbidimetric immunoassay to contain increased levels of **carbohydrate-deficient transferrin** were analyzed.

L14 ANSWER 13 OF 57 CAPLUS COPYRIGHT 2005 ACS on STN

2003:652705 Document No. 140:57176 Biochemical markers of drinking.

Yamauchi, Masayoshi; Searashi, Yasuyuki; Inoue, Takahiro (Dep. of Internal Medicine, Kinugasa Hospital, Japan). Rinsho Kensa, 47(6), 599-606 (Japanese) 2003. CODEN: RNKNAT. ISSN: 0485-1420. Publisher: Igaku Shoin Ltd..

AB A review on biol. indicators for alc. drinking and alc. liver disease. The biol. indicators discussed are γ-GTP, alanine and aspartate aminotransferase ratio, glutamate dehydrogenase and ornithine carbamyl transferase ratio, serum **carbohydrate-deficient transferrin**, serum lysosomal enzymes, L-fucose concentration in urine, hepatic fibrotic markers, cytokines and serum adhesion mols., serum **antibodies** to alc. hepatocyte membrane and acetaldehyde adducts.

L14 ANSWER 14 OF 57 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 4

2003:361856 Document No.: PREV200300361856. Development and evaluation of a new **carbohydrate-deficient transferrin** (CDT)-specific monoclonal **antibody.** Althaus, H. [Reprint Author]; Hackler, R.; Fischer, B. [Reprint Author]; Schaefer, J. R.; Walter, G. [Reprint Author]; Harthus, H. P. [Reprint Author]. Dade Behring Marburg GmbH, Marburg, Germany. Clinical Chemistry, (June 2003) Vol. 49, No. S6, pp. A113. print.
Meeting Info.: 55th Annual Meeting of the AACC (American Association for Clinical Chemistry). Philadelphia, PA, USA. July 20-24, 2003. American Association for Clinical Chemistry.
CODEN: CLCHAU. ISSN: 0009-9147. Language: English.

L14 ANSWER 15 OF 57 MEDLINE on STN DUPLICATE 5

2002698680. PubMed ID: 12446474. **Carbohydrate-deficient transferrin** isoforms measured by capillary zone electrophoresis for detection of alcohol abuse. Legros Franz J; Nuyens Vincent; Minet Eddy; Emonts Philippe; Boudjeltia Karim Zouaoui; Courbe Anne; Ruelle Jean-Luc; Colicis Jacques; de L'Escaille Francois; Henry Jean-Pol. (Laboratory of Experimental Medicine, Universite Libre de Bruxelles and Centre Hospitalier Universitaire Andre Vesale, 706, route de Gozee, B6110 Montigny-le-Tilleul, Belgium.. franz.legros@chu-charleroi.be) . Clinical chemistry, (2002 Dec) 48 (12) 2177-86. Journal code: 9421549. ISSN: 0009-9147. Pub. country: United States. Language: English.

AB BACKGROUND: Measurements of **carbohydrate-deficient transferrin** (CDT) are used as markers of alcohol abuse. We developed a capillary zone electrophoresis (CZE) method aimed at improving accuracy of CDT testing. METHODS: We studied 111 alcohol abusers with Alcohol Use Disorders Identification Test scores >11 and 50 teetotalers. CZE was performed with a P/ACE 5500, fused-silica capillaries, and a CEofix CDT reagent set. After iron saturation, sera were loaded by low-pressure injection, separated at 28 kV, and monitored at 214 nm. We identified the transferrin isoforms by migration times, treatment with 100 U/L neuraminidase, and immunosubtraction with anti-human transferrin and anti-C-reactive protein **antibodies.** We compared CZE results with current biological markers of alcohol abuse, including the %CDT turbidimetric immunoassay. RESULTS: Migration times of the isoforms were identical in both populations. Asialotransferrin was missing in teetotalers but present in 92% of alcohol abusers. Disialotransferrin was higher in those who consumed excessive amounts of alcohol, whereas mean trisialotransferrin concentration was not affected by alcohol abuse. At cutoffs to maximize sensitivity and specificity, these values were 0.92 and 1 [mean ROC area (MRa), 0.96; 95% confidence interval (CI), 0.93-0.99] for asialotransferrin; 0.84 and 0.94 for the sum of asialo- + disialotransferrin (MRa, 0.94; 95% CI, 0.91-0.98); 0.79 and 0.94 for disialotransferrin (MRa, 0.89; 95% CI, 0.84-0.94); 0.62 and 0.53 for trisialotransferrin (MRa, 0.58; 95% CI, 0.49-0.68); 0.79 and 0.82 for a 3% %CDT; and 0.83 and 0.69 for a 2.6% cutoff (MRa, 0.87; 95% CI, 0.81-0.92). Current markers lack sensitivity (<0.65). Transferrins were not significantly correlated with serum enzymes and mean erythrocyte volume. CONCLUSIONS: CZE-isolated desialylated transferrin isoforms allowed differentiation between chronic alcohol abusers and teetotalers.

L14 ANSWER 16 OF 57 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:554973 The Genuine Article (R) Number: 566UR. Alcohol abstinence does not offset the strong negative effect of lifetime alcohol consumption on the outcome of interferon therapy. Tabone M (Reprint); Sidoli L; Laudi C; Pellegrino S; Rocca G; Della Monica P; Fracchia M; Galatola G; Molinaro G C; Arico S; Pera A. Osped Mauriziano Umberto 1, Div Gastroenterol, Largo Turati 62, I-10128 Turin, Italy (Reprint); Mauriziano Hosp, Gastroenterol Unit, Turin, Italy; Mauriziano Hosp, Cent Lab, Turin, Italy; Osped Molinette, Dept Gastroenterol, Turin, Italy. JOURNAL OF VIRAL HEPATITIS (JUL 2002) Vol. 9, No. 4, pp. 288-294. ISSN: 1352-0504. Publisher: BLACKWELL PUBLISHING LTD, 9600 GARSINGTON RD, OXFORD OX4 2DG, OXON, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Heavy alcohol consumption has been reported to negatively affect the outcome of interferon therapy. We studied the impact of lifetime alcohol consumption in patients with chronic hepatitis C treated with interferon after 6 months of alcohol withdrawal. Alcohol intake was measured when patients with chronic hepatitis C were referred to us for the first time, and from that moment complete abstinence was recommended. After 6 months of abstinence, 150 patients with persistent elevated serum alanine aminotransferase (ALT) have been treated with interferon (IFN)-alpha, 3 or 6 summaryU three times per week for 12 months. Univariate and multivariate analysis were performed to identify the predictors of treatment response. **Carbohydrate-deficient transferrin** was employed to assess alcoholic abstinence. The sustained response rate felt from 33% in nondrinkers to 20% of mild-drinkers and to only 9% in heavy drinkers. Drinker patients showed a relapse rate twice as high as that of nondrinkers. According to the multivariate analysis, the strongest independent predictors of nonresponse were genotype 1b infection, age of the patients and their lifetime alcohol intake. **Carbohydrate-deficient transferrin** detected at baseline, at 3 months of therapy and at the end of follow-up gave a positive result only in eight determinations (1.77%), confirming the compliance of patients to our recommendation of alcohol abstinence. Lifetime alcohol consumption has a strong negative effect on the outcome of interferon treatment, mainly in heavy drinkers. A 6-month period of abstinence may not be sufficient to offset this negative effect on treatment outcome.

L14 ANSWER 17 OF 57 MEDLINE on STN DUPLICATE 6

2002450452. PubMed ID: 12198372. Acetaldehyde-induced growth retardation and micro-heterogeneity of the sugar chain in transferrin synthesized by HepG2 cells. Searashi Yasuyuki; Yamauchi Masayoshi; Sakamoto Kazuhiko; Ohata Mitsuru; Asakura Tadashi; Ohkawa Kiyoshi. (Division of Gastroenterology and Hepatology, Jikei University School of Medicine, Tokyo, Japan.. searashi@pj8.so-net.ne.jp) . Alcoholism, clinical and experimental research, (2002 Aug) 26 (8 Suppl) 32S-37S. Journal code: 7707242. ISSN: 0145-6008. Pub. country: United States. Language: English.

AB BACKGROUND: A **carbohydrate-deficient transferrin** (CDT) is the most useful marker of alcohol abuse; however, the mechanism of production and the pathophysiologic roles of CDT remain obscure. The effects of alcohol and its metabolites on growth and proliferation, transferrin synthesis, and phosphomannomutase enzyme activity in a human hepatoblastoma, HepG2, were examined. METHODS: HepG2 cells were treated with either ethanol at 80 mM or acetaldehyde at 400 microM. Transferrin secreted by the cells was prepared from conditioned culture medium by single-step immunoaffinity column chromatography using a goat-specific **antibody** against human transferrin. Phosphomannomutase and some related enzyme activities in the cell extracts were determined. Reverse transcription-polymerase chain reaction analysis of phosphomannomutase mRNA expression was also determined in HepG2 cultured with or without acetaldehyde (400 microM). RESULTS: HepG2 cells usually synthesized and secreted transferrin with three separated bands:

main broad bands estimated to be 78 to 82 kDa, 75 kDa, and 72 kDa. The last two bands were compatible with part or the entire N-glycans-deficient transferrin (CDT) from alcoholic liver damage. Increased secretion of CDT from HepG2 correlated well with the extent of growth retardation to the level of confluent cell density. The activity of phosphomannomutase also decreased with prolongation of cellular doubling time. Furthermore, acetaldehyde treatment at 400 microM accelerated the inhibitory effect of cell growth compared with nontreated cells, and this condition facilitated CDT secretion from HepG2 cells. Determination of the enzyme activity and mRNA expression indicated that acetaldehyde showed competitive type inhibition of phosphomannomutase activity but not suppression of phosphomannomutase gene expression. CONCLUSIONS: By culturing HepG2 cells with acetaldehyde containing media, growth inhibition-dependent increase of CDT showed good correlation with reduced enzyme activity of phosphomannomutase. Acetaldehyde facilitated growth retardation, inhibition of phosphomannomutase activity, and increased secretion of CDT. The HepG2 cell line is useful as an in vitro model to investigate the pathophysiologic state of alcoholic liver damage and mechanisms of production as well as the physiologic role of CDT.

L14 ANSWER 18 OF 57 CAPLUS COPYRIGHT 2005 ACS on STN

2002:713613 Document No. 137:364746 Acetaldehyde-induced growth retardation and microheterogeneity of the sugar chain in transferrin synthesized by HepG2 cells. Searashi, Yasuyuki; Yamauchi, Masayoshi; Sakamoto, Kazuhiko; Ohata, Mitsuru; Asakura, Tadashi; Ohkawa, Kiyoshi (Division of Gastroenterology and Hepatology, Jikei University School of Medicine, Tokyo, Japan). Alcoholism: Clinical and Experimental Research, 26(8, Suppl.), 32S-37S (English) 2002. CODEN: ACRSDM. ISSN: 0145-6008. Publisher: Lippincott Williams & Wilkins.

AB Background: A **carbohydrate-deficient transferrin** (CDT) is the most useful marker of alc. abuse; however, the mechanism of production and the pathophysiol. roles of CDT remain obscure. The effects of alc. and its metabolites on growth and proliferation, transferrin synthesis, and phosphomannomutase enzyme activity in a human hepatoblastoma, HepG2, were examined Methods: HepG2 cells were treated with either ethanol at 80 mM or acetaldehyde at 400 μM. Transferrin secreted by the cells was prepared from conditioned culture medium by single-step immunoaffinity column chromatog. using a goat-specific **antibody** against human transferrin. Phosphomannomutase and some related enzyme activities in the cell exts. were determined Reverse transcription-polymerase chain reaction anal. of phosphomannomutase mRNA expression was also determined in HepG2 cultured with or without acetaldehyde (400 μM). Results: HepG2 cells usually synthesized and secreted transferrin with three separated bands: main broad bands estimated to be 78 to 82 kDa, 75 kDa, and 72 kDa. The last two bands were compatible with part or the entire N-glycans-deficient transferrin (CDT) from alc. liver damage. Increased secretion of CDT from HepG2 correlated well with the extent of growth retardation to the level of confluent cell d. The activity of phosphomannomutase also decreased with prolongation of cellular doubling time. Furthermore, acetaldehyde treatment at 400 μM accelerated the inhibitory effect of cell growth compared with nontreated cells, and this condition facilitated CDT secretion from HepG2 cells. Determination of the enzyme activity and mRNA expression indicated that acetaldehyde showed competitive type inhibition of phosphomannomutase activity but not suppression of phosphomannomutase gene expression. Conclusions: By culturing HepG2 cells with acetaldehyde containing media, growth inhibition-dependent increase of CDT showed good correlation with reduced enzyme activity of phosphomannomutase. Acetaldehyde facilitated growth retardation, inhibition of phosphomannomutase activity, and increased secretion of CDT. The HepG2 cell line is useful as an in vitro model to investigate the pathophysiol. state of alc. liver damage and mechanisms of production as well as the physiol. role of CDT.

L14 ANSWER 19 OF 57 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights

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2002266690 EMBASE Detection and identification of protein variants and adducts in blood and tissues: An application of soft ionization mass spectrometry to clinical diagnosis. Shimizu A.; Nakanishi T.; Kishikawa M.; Miyazaki A.. A. Shimizu, Department of Clinical Pathology, Osaka Medical College, 2-7 Daigakucho, Takatsuki City, Osaka 569-8686, Japan. shimizu@poh.osaka-med.ac.jp. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences Vol. 776, No. 1, pp. 15-30 25 Aug 2002.
Refs: 45.
ISSN: 1570-0232. CODEN: JCBAAI
S 1570-0232(02)00031-4. Pub. Country: Netherlands. Language: English. Summary Language: English.

ED Entered STN: 20020808

AB The detection and identification of protein variants and abnormally increased modified proteins are important for clinical diagnosis. We applied soft ionization mass spectrometry (MS) to analyze proteins in blood and tissues from various patients. Over the past 8 years, we diagnosed 132 cases (55 kinds) of variant proteins including hemoglobin (Hb), transthyretin (TTR), and Cu/Zn-superoxide dismutase (SOD-1), using MS as the leading technology. Of these variants, eight were new, and nine were the first cases in Japan. Some abnormal Hb cause diseases, and most of them cause erroneous levels of glycated Hb, HbA1c, i.e., a popular index of diabetes. Most of the variant TTR causes amyloidotic polyneuropathy. Variant SOD-1 causes amyotrophic lateral sclerosis. We first showed that immunoprecipitation by a specific antiserum is a reliable and simple method to prepare protein from sera and tissues for analysis by matrix-assisted laser desorption time-of-flight MS, and liquid chromatography-electrospray ionization MS (LC-ESI-MS). The use of this technology has become widespread. Using an immunoprecipitated target protein and LC-ESI-MS, we showed that the ratios of tetra-, di- and a-sialo-transferrin from two cases of congenital glycoprotein deficient syndrome were clearly distinguishable from those of control samples. We first reported a unique modified form of TTR, that is, S-sulfonated TTR, which increased markedly and specifically in three cases with molibdenum cofactor deficiency. We proposed that S-sulfonated TTR is a useful marker for screening this disease. ESI-MS was successfully used for the accurate determination of HbA1c, and we clarified the extent of discrepancies between the HbA1c value measured by conventional methods and the accurate values for samples containing various Hb variants determined by the MS method.

L14 ANSWER 20 OF 57 CAPLUS

2001:186023 Document No. 134:219338 Systems including an immunoaffinity cartridge and a preconcentrator cartridge and a mass spectrometer for detecting analytes. Naylor, Stephen; O'brien, John F.; Bergen, H. Robert, III (USA). PCT Int. Appl. WO 2001018540 A1 20010315, 40 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US24602 20000908. PRIORITY: US 1999-391432 19990908.

AB Systems for detecting analytes that include an immunoaffinity cartridge, a preconcentrator cartridge, and a mass spectrometer are described. The system also can include a membrane cartridge. Methods for detecting the presence or absence of an analyte in a biol. sample also are described. Serum samples of patients with carbohydrate-deficient glycoprotein syndrome (CDGS) (both phosphomannomutase- and phosphomannoisomerase-deficient) and of chronic alcoholics were analyzed using a system with an immunoaffinity cartridge having immobilized rabbit antitransferrin **antibodies**, a preconcentrator cartridge and an electrospray

ionization mass spectrometer. Transferrin immunopurified from the CDGS serum revealed three distinct species at 79561, 77353, and 75145 Da; while that from the chronic alcoholics showed two species at 79561 and 77353 Da. In normal serum, only a single ion is detected at 79561 Da.

L14 ANSWER 21 OF 57 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:655776 The Genuine Article (R) Number: 459TY. Rapid and simple single nanogram detection of glycoproteins in polyacrylamide gels and on electroblots. Steinberg T H; Top K P O; Berggren K N; Kemper C; Jones L; Diwu Z J; Haugland R P; Patton W F (Reprint). Mol Probes Inc, Proteom Sect, 4849 Pitchford Ave, Eugene, OR 97402 USA (Reprint); Mol Probes Inc, Proteom Sect, Eugene, OR 97402 USA. PROTEOMICS (JUL 2001) Vol. 1, No. 7, pp. 841-855. ISSN: 1615-9853. Publisher: WILEY-V C H VERLAG GMBH, PO BOX 10 11 61, D-69451 BERLIN, GERMANY. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The fluorescent hydrazide, Pro-Q Emerald 300 dye, may be conjugated to glycoproteins by a periodic acid Schiff's (PAS) mechanism. The glycols present in glycoproteins are initially oxidized to aldehydes using periodic acid. The dye then reacts with the aldehydes to generate a highly fluorescent conjugate. Reduction with sodium metabisulfite or sodium borohydride is not required to stabilize the conjugate. Though glycoprotein detection may be performed on transfer membranes, direct detection in gels avoids electroblotting and glycoproteins may be visualized within 2-4 h of electrophoresis. This is substantially more rapid than PAS labeling with digoxigenin hydrazide followed by detection with an antidigoxigenin **antibody** conjugate of alkaline phosphatase, or PAS labeling with biotin hydrazide followed by detection with horseradish peroxidase or alkaline phosphatase conjugates of streptavidin, which require more than eight hours to complete. Pro-Q Emerald 300 dye-labeled gels and blots may be post-stained with SYPRO Ruby dye, allowing sequential two-color detection of glycosylated and nonglycosylated proteins. Both fluorophores are excited with mid-range UV illumination. Pro-Q Emerald 300 dye maximally emits at 530 nm (green) while SYPRO Ruby dye maximally emits at 610 nm (red), As little as 300 pg of alpha1-acid glycoprotein (40% carbohydrate) and 1 ng of glucose oxidase (12% carbohydrate) or avidin (7% carbohydrate) are detectable in gels after staining with Pro-Q Emerald 300 dye. Besides glycoproteins, as little as 2-4 ng of lipopolysaccharide is detectable in gels using Pro-Q Emerald 300 dye while 250-1000 ng is required for detection with conventional silver staining. Detection of glycoproteins may be achieved in sodium dodecyl sulfate-polyacrylamide gels, two-dimensional gels and on polyvinylidene difluoride membranes.

L14 ANSWER 22 OF 57 MEDLINE on STN

2001159945. PubMed ID: 11238305. Rapid determination of transferrin isoforms by immunoaffinity liquid chromatography and electrospray mass spectrometry. Lacey J M; Bergen H R; Magera M J; Naylor S; O'Brien J F. (Biochemical Genetics Laboratory, Department of Laboratory Medicine and Pathology, Biomedical Mass Spectrometry and Functional Proteomics Facility, Mayo Clinic, 200 First St. SW, Rochester, MN 55905, USA.) Clinical chemistry, (2001 Mar) 47 (3) 513-8. Journal code: 9421549. ISSN: 0009-9147. Pub. country: United States. Language: English.

AB BACKGROUND: Congenital disorders of glycosylation (CDG) are autosomal recessive disorders that produce increased serum **carbohydrate-deficient transferrin** (CDT) isoforms. Methods to resolve CDT from fully glycosylated transferrin (Trf) have been based on a neutral shift in the isoelectric focusing (IEF) pattern or on a reduction in the negative charge, allowing resolution by anion-exchange chromatography. Our purpose was to develop a method of resolution and relative quantification of Trf isoforms using online immunoaffinity liquid chromatography-mass spectrometry (LC-MS). METHODS: Serum (25 microL) was diluted with 100 microL of water before application to an immunoaffinity column that sequestered Trf isoforms. Trf isoforms were eluted from the immunoaffinity column, concentrated on a C4 column, eluted from the C4

column, and introduced into the mass spectrometer. Analysis of the Trf isoforms was entirely automated and completed in <10 min per sample. RESULTS: The LC-MS method demonstrated that the major abnormal Trf isoforms in CDG lack one complete oligosaccharide structure (mono-oligosaccharide) or both oligosaccharide structures (a-oligosaccharide), but not the sialic acids, as presumed on the basis of IEF methods. Calculation of relative ratios among three possible species (mono-/di-oligosaccharide and a-/di-oligosaccharide) is reproducible [mean intra- and interassay CVs were 9.3% (n = 10) and 10% (n = 5), respectively]. A reference range for patients <18 years was determined by analysis of 209 samples (for mono-/di-oligosaccharide, the median was 0.041 and the range was 0.018-0.083; for a-/di-oligosaccharide, the median was 0.007 and the range was 0.002-0.036). Comparison of data obtained with an affinity chromatography-IEF method and the LC-MS method demonstrated equivalence in the interpreted results (n = 170). CONCLUSIONS: Advantages of the LC-MS method include improved sensitivity, minimal sample preparation, and an analysis time of <10 min. The method was automated, which allowed high throughput, with >100 samples analyzed in a single day. Moreover, the nature of the oligosaccharide defect in CDG is accurately reflected by mass resolution, and subtle oligosaccharide truncations may also be detected by this method.

L14 ANSWER 23 OF 57 EMBASE

2001202976 EMBASE Neuropeptides in the duodenal mucosa of chronic alcoholic heavy drinkers. Hauge T.; Persson J.; Sjolund K.. T. Hauge, Division of Gastroenterology, Department of Internal Medicine, Ostfold Central Hospital, N-1603 Fredrikstad, Norway. Alcohol and Alcoholism Vol. 36, No. 3, pp. 213-218 2001.
Refs: 25.
ISSN: 0735-0414. CODEN: ALALDD
Pub. Country: United Kingdom. Language: English. Summary Language: English.
ED Entered STN: 20010710

AB Previous studies have shown that patients with chronic alcohol ingestion may show a variety of morphological and functional alterations in the small intestine. In this study, we have focused on the neuroendocrine system in the duodenal mucosa in chronic alcoholics; an area little studied. Twenty-three defined chronic alcoholics admitted to the hospital for detoxification underwent clinical examination, followed by upper gastrointestinal endoscopy and blood tests on average 4 days after the most recent alcohol intake. Biopsy specimens were taken from the distal part of the descending duodenum for both immunohistochemical and routine histological examination. The control group consisted of 25 patients referred for upper endoscopy mainly because of dyspepsia (ulcer, reflux type), but who were otherwise healthy. A normal **carbohydrate-deficient transferrin** and a history of low alcohol consumption (<40 g/week) were required for inclusion in the control group. The tissue specimens were studied using antisera for the following neuropeptides: cholecystokinin, galanin, gastric inhibitory peptide (GIP), glucagon, motilin, neuropeptide Y, pituitary adenylyl cyclase activating peptide, secretin, serotonin, somatostatin, substance P, vasoactive intestinal polypeptide and protein gene product, as a general marker for neurones and cells of the diffuse neuroendocrine system. The density of nerve fibres was evaluated semi-quantitatively and the number of endocrine cells per unit length of mucosa was counted in sections cut perpendicularly to the mucosal surface. All the different peptidergic nerve fibres in the alcohol group showed higher densities than the corresponding controls. However, this was not a statistically significant difference. A slightly significant increase (P = 0.02) in the numbers of glucagon and GIP cells was seen in the alcohol group. Gastrointestinal symptoms were frequently present (87%) in chronic alcoholics. We suggest that chronic alcohol consumption in man may have a general effect on the peptidergic nerve system and some endocrine cell types in the duodenal mucosa.

L14 ANSWER 24 OF 57 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

2002063923 EMBASE Plasma sialic-acid index of apolipoprotein J (SIJ): A new alcohol intake marker. Ghosh P.; Hale E.A.; Lakshman M.R.. M.R. Lakshman, George Washington Univ. Med. Center, Washington, DC 20037, United States. rlax@erols.com. Alcohol Vol. 25, No. 3, pp. 173-179 2001.
Refs: 17.
ISSN: 0741-8329. CODEN: ALCOEX
S 0741-8329(01)00187-2. Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20020301

AB Although plasma **carbohydrate-deficient transferrin** (CDT) is considered a viable biochemical marker for chronic alcohol consumption, it is valid only when an individual's daily alcohol consumption exceeds 60 g. In addition, it is less sensitive in women drinkers than in men drinkers. We have established that chronic alcohol consumption impairs the hepatic sialylation of a number of glycoproteins by specifically down-regulating Gal-β-1,4GlcNAc α2,6-sialyltransferase mRNA. Significantly, we found that chronic ethanol consumption markedly inhibits hepatic sialylation of apolipoprotein J (Apo J), a 70-kDa N-glycosylated protein of plasma HDL. Because the sialic-acid index of Apo J (SIJ; moles of sialic acid per mole of Apo J protein) is approximately seven times more than that for transferrin (28 vs. 4), we have evaluated whether plasma SIJ would be an even more sensitive marker for chronic ethanol consumption than CDT in both rats and human subjects. The method involves immunoaffinity purification of plasma HDL-Apo J, followed by its sialic acid determination. We have found that chronic ethanol feeding resulted in loss of sialic acid residues of plasma HDL-Apo J in rats. This loss of sialic acid was positively correlated with both amount and duration of ethanol treatment. In human subjects, an intake of about 60 g of alcohol for 30 days led to almost 50% ($P < .01$) depletion of sialic acid from plasma HDL-Apo J. Further, we established that there was a positive correlation of alteration in SIJ with alcohol consumption, detoxification, abstinence, and relapse in human alcohol-dependent patients (sensitivity, 90%-92%). In addition, plasma SIJ was decreased by 50%-57% ($P < .01$) in both male and female alcohol-dependent subjects. We suggest that plasma SIJ can be used as a viable marker for early detection of chronic alcohol consumption in human beings.

L14 ANSWER 25 OF 57 CAPLUS COPYRIGHT 2005 ACS on STN

2000:421411 Document No. 133:40212 Dipstick for carbohydrate-free transferrin assay. Sundrehagen, Erling (Axis-Shield Asa, Norway; Dzieglewska, Hanna). PCT Int. Appl. WO 2000036418 A1 20000622, 50 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB4191 19991210. PRIORITY: GB 1998-27411 19981211.

AB The invention relates to a new dipstick assay for detecting and quantifying the content of an analyte in a sample. The assay is particularly useful for example in the diagnosis and monitoring of alcoholism by the detection of asialo transferrin or carbohydrate free transferrin (CFT). Thus, provided is a dipstick for determining the content of a target analyte variant in a mixture of analyte variants in a sample, comprising: (a) a sample application zone, (b) a screening zone having an immobilized binding ligand having a binding affinity for a non-target analyte variant or variants, (c) a conjugate zone comprising a detector reagent, (d) a reading zone for detection of said analyte. A dipstick for

determination of CFT in serum had a zone of immobilized Sambuccus nigra lectin and
ConA above the sample application zone, a detector reagent zone with anti-transferrin **antibody** labeled with blue latex particles, and a reading zone containing immobilized anti-transferrin **antibodies.** An adsorbent sink pad was at the far end of the dipstick.

L14 ANSWER 26 OF 57 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2000:883949 The Genuine Article (R) Number: 374XU. Detecting alcoholic relapse posttransplant. Jowsey S G (Reprint). Mayo Clin, Mayo Bldg W 11A, 200 1st St SW, Rochester, MN 55905 USA (Reprint); Mayo Clin, Rochester, MN 55905 USA. LIVER TRANSPLANTATION (NOV 2000) Vol. 6, No. 6, pp. 812-814. ISSN: 1527-6465. Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Background: The role of **carbohydrate-deficient transferrin** (CDT) as a reliable marker for the detection of chronic alcohol abuse has been discussed controversially.

Methods: Therefore, we investigated CDT in the sera from 405 subjects with different alcohol intake. Besides healthy control subjects (n = 42), inpatients and outpatients in a department of gastroenterology (n = 325) and patients admitted to a department of otorhinolarynology (n = 38) were studied. A total of 213 patients suffered from various forms of liver diseases, and 89 patients had liver transplantation. CDT values were determined by a double-**antibody** radioimmunoassay.

Results In the 241 alcohol-abstinent subjects, CDT levels ranged from 3 to 90 units L-1 (median = 12); the 92 moderate drinkers (20-60 g of alcohol per day) showed values from 3 to 40 units L-1 (median = 12), and the 72 subjects with chronic alcohol abuse (> 60 g per day) revealed CDT levels from 3 to 100 units L-1 (median = 16). The diagnostic specificity for alcohol abuse was 86.8% for men (sensitivity 36.9%) and 95% for women (sensitivity 0%).

Conclusion: Our data indicate that measurement ofCDT does not reach clinical use in the detection of chronic alcohol abuse in an unselected population because of its insufficient specificity and sensitivity.

L14 ANSWER 27 OF 57 MEDLINE on STN DUPLICATE 7
2001310505. PubMed ID: 11383548. Glycosylation of transferrin in Alzheimer's disease and alcohol-induced dementia. van Rensburg S J; Berman P A; Potocnik F C; Taljaard J J. (Department of Chemical Pathology, Tygerberg Hospital and University of Stellenbosch Medical School, South Africa.. sjvr@gerga.sun.ac.za) . Metabolic brain disease, (2000 Dec) 15 (4) 243-7. Journal code: 8610370. ISSN: 0885-7490. Pub. country: United States. Language: English.
AB Transferrin is a glycosylated metal-binding serum protein. **Carbohydrate-deficient transferrin** (CDT) is a marker of recent and heavy alcohol intake. A genetic variant of transferrin, TfC2, occurs with increased frequency in patients with Alzheimer's disease (AD). Hence the question arose whether, in addition to an altered amino acid sequence, there could also be a difference in the glycosylation state of transferrin in patients with dementia. Serum samples of 37 AD and 13 Alcohol-induced dementia patients as well as 10 healthy controls were analyzed for abnormal Tf variants, using isoelectric focusing followed by blotting with anti-Tf **antibodies.** This allowed the direct visualization of glycosylation variants of transferrin, and assessment of any increase in underglycosylated forms (di-, mono- and asialo transferrin).

L14 ANSWER 28 OF 57 MEDLINE on STN DUPLICATE 8
2000149946. PubMed ID: 10684785. Cytochrome P-450 2E1 activity and oxidative stress in alcoholic patients. Dupont I; Bodenez P; Berthou F; Simon B; Bardou L G; Lucas D. (Faculte de Medecine de Brest, EA 948, Laboratoire de Biochimie, BP 815, 29285 Brest, and Service d'Alcoologie,

Hopital de Bohars, CHU de Brest, France.) Alcohol and alcoholism (Oxford, Oxfordshire), (2000 Jan) 35 (1) 98-103. Journal code: 8310684. ISSN: 0735-0414. Pub. country: ENGLAND: United Kingdom. Language: English.

AB As cytochrome P-450 2E1 (CYP2E1) induction was related to oxidative stress in experimental models, the aim of this study was to investigate the relationship between CYP2E1 activity and markers of oxidative stress in 40 alcoholic patients entering a rehabilitation programme. Plasma oxidized proteins, lipid peroxides (LPO) and **antibodies** against hydroxyethyl radical (HER) or malondialdehyde (MDA) adducts were assessed as markers of the production of free radicals, whereas vitamin E levels were evaluated as a marker of the antioxidant defence. CYP2E1 activity was determined by using the 6-hydroxychlorzoxazone:chlorzoxazone blood metabolic ratio, 2 h after drug intake. This ratio was increased by 4-fold in alcoholics, compared to non-alcoholic patients, and was correlated with daily intake of ethanol, **carbohydrate-deficient transferrin**, and blood alcohol level at the time of admission to hospital. Plasma levels of LPO and oxidized proteins were slightly increased (20%) in alcoholic patients when compared with the control group, whereas those of vitamin E were found to be slightly decreased (by 18%). **Antibodies** against HER or MDA adducts showed a very significant increase. However, when alcoholic patients were divided into two groups according to low or high CYP2E1 induction, no significant difference was observed in the variation of these parameters, except for anti-HER adducts **antibodies.** Therefore, our study confirms the main involvement of CYP2E1 in HER production. By contrast, CYP2E1 does not appear to be the main factor responsible for the oxidative stress occurring during human chronic alcoholism. Free radicals from other sources may therefore contribute significantly to the generation of this oxidative stress.

L14 ANSWER 29 OF 57 CAPLUS COPYRIGHT 2005 ACS on STN

1999:42622 Document No. 130:91547 Assay for carbohydrate-free transferrin. Sundrehagen, Erling (Axis Biochemicals Asa, Norway; Dzieglewska, Hanna, E.). PCT Int. Appl. WO 9900672 A1 19990107, 39 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-GB1875 19980626. PRIORITY: GB 1997-13559 19970626.

AB The present invention provides a method for the determination of carbohydrate-free transferrin in a body fluid for use in the assessment of alc. consumption, said method comprising (a) contacting a sample of said body fluid with a carbohydrate-binding ligand, to bind any carbohydrate or carbohydrate-containing moieties in said sample to said ligand; (b) separating a fraction not binding to said ligand and (c) determining the content of transferrin in said fraction. Also provided are kits for carrying out such a method.

L14 ANSWER 30 OF 57 MEDLINE on STN DUPLICATE 9

1999402864. PubMed ID: 10471642. Microheterogeneity of serum glycoproteins in patients with chronic alcohol abuse compared with carbohydrate-deficient glycoprotein syndrome type I. Henry H; Froehlich F; Perret R; Tissot J D; Eilers-Messerli B; Lavanchy D; Dionisi-Vici C; Gonvers J J; Bachmann C. (Central Clinical Chemistry Laboratory, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland.. Hugues.Henry@chuv.hospvd.ch) . Clinical chemistry, (1999 Sep) 45 (9) 1408-13. Journal code: 9421549. ISSN: 0009-9147. Pub. country: United States. Language: English.

AB BACKGROUND: Chronic alcohol abuse alters the normal N-glycosylation of transferrin, producing the **carbohydrate-deficient**

transferrin isoforms. This alteration could be similar to that present in patients with carbohydrate-deficient glycoprotein syndrome type 1 (CDG1). We thus compared the alterations of N-glycans present in patients with alcoholism and patients with CDG1. METHODS: The N-glycans of serum glycoproteins were compared in sera of patients with alcoholism, patients with CDG1, and controls by two-dimensional electrophoresis, neuraminidase, peptide:N-glycosidase F, and endoglycosidase F2 treatments. A specific **antibody** directed against the amino acid sequence surrounding the N-432 N-glycosylation site of transferrin was prepared (SZ-350 **antibody**). RESULTS: In patients with alcoholism, the abnormal transferrin and alpha(1)-antitrypsin isoforms were devoid of a variable number of entire N-glycan moieties and were identical with those present in CDG1. In the serum of patients with alcoholism, this finding was less pronounced than in CDG1. In contrast to CDG1, there was no decrease in clusterin or serum amyloid P in patients with alcoholism. The SZ-350 **antibody** recognized only transferrin isoforms with one or no N-glycan moieties. CONCLUSION: **Antibodies** directed against specific N-glycosylation sites of glycoproteins could be useful for developing more specific immunochemical tests for the diagnosis of chronic alcohol abuse.

L14 ANSWER 31 OF 57 MEDLINE on STN DUPLICATE 10

1999231503. PubMed ID: 10217151. Structural studies on sugar chains of **carbohydrate-deficient transferrin** from patients with alcoholic liver disease using lectin affinity electrophoresis. Inoue T; Yamauchi M; Ohkawa K. (Department of Internal Medicine (I), The Jikei University School of Medicine, Tokyo, Japan.. ramses@mxr.meshnet.or.jp) . Electrophoresis, (1999 Mar) 20 (3) 452-7. Journal code: 8204476. ISSN: 0173-0835. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB It is well-known that microheterogeneity of human serum transferrin observed in alcoholics manifests as sialic acid-deficient transferrin isoforms, otherwise known as **carbohydrate-deficient transferrin** (CDT). A recent study demonstrated that serum CDT lacked one or both of the entire carbohydrate chains but the investigation required several troublesome procedures. The aim of the present study was to confirm the sugar chain structures of serum transferrin, and of serum CDT in particular, from patients with alcoholic liver disease (ALD) using conventional lectin affinity electrophoresis which might be useful in the clinical setting. The serum CDT obtained from ALD-patients was partially purified using an anion exchanger. Serum transferrin and the partially purified serum CDT were investigated by concanavalin A (Con A)- and Datura stramonium agglutinin (DSA)-affinity electrophoresis followed by **antibody**-affinity blotting and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Western blotting. By Con A-affinity electrophoresis, serum CDT was separated into weakly reactive and nonreactive transferrins which showed slower electrophoretic mobilities than those from the healthy controls. Moreover, nearly all of the serum CDT was nonreactive with DSA. On SDS-PAGE, the molecular masses of serum CDT were estimated to be approximately 75 and 72 kDa, which corresponded to those of partially and completely deglycosylated transferrin obtained from the healthy controls (78 kDa), respectively. In conclusion, these results indicated that the sugar chain structures of serum CDT from patients with ALD show not merely a loss of terminal sialic acids, but also the absence of asparagine-N-linked oligosaccharides.

L14 ANSWER 32 OF 57 MEDLINE on STN DUPLICATE 11

1999151684. PubMed ID: 10029226. Liver function in workers exposed to N,N-dimethylformamide during the production of synthetic textiles. Wrbitzky R. (Institute and Outpatient Clinic for Occupational, Social, and Environmental Medicine, Erlangen, Germany.. Renate.Wrbitzky@rzmail.uni-erlangen.de) . International archives of occupational and environmental health, (1999 Jan) 72 (1) 19-25. Journal code: 7512134. ISSN: 0340-0131. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB OBJECTIVE: In a factory producing synthetic fibers the hepatotoxic effects

of the solvent N,N-dimethylformamide (DMF) were investigated in 126 male employees, especially with regard to the combination effects of DMF exposure and ethyl alcohol consumption. A collective of similar structure from the same factory served as a control collective. METHODS: Reference is made to the results of air measurements and biological monitoring presented in a previous publication. The DMF concentrations in the air ranged from < 0.1 (detection limit) to 37.9 ppm (median 1.2 ppm). Concentrations of the DMF metabolite N-methylformamide (NMF) in urine were 0.05-22.0 mg/l (preshift) and 0.9-100.0 mg/l (postshift), corresponding to 0.02-44.6 mg/g creatinine (preshift) and 0.4-62.3 mg/g creatinine (postshift). A standardized anamnesis was drawn up for relevant previous illnesses and other factors influencing liver function. The laboratory tests included parameters especially relevant to the liver (e.g., AST, ALT, gamma-GT, hepatitis B and C **antibodies**, and **carbohydrate-deficient transferrin**). RESULTS: The results indicate a statistically significant toxic influence of DMF on liver function. Alcohol has a synergistic effect. The effects of DMF and those of alcohol are dose-dependent. Under the existing workplace conditions the hepatotoxic effects of alcohol are more severe than those of DMF. In the exposed group there was a statistically significantly greater number of persons who stated that they had drunk less since the beginning of exposure (13% versus 0). This corresponded with the data on symptoms occurring after alcohol consumption (71% versus 4%). In the work areas with lower-level exposure to DMF there was greater alcohol consumption. It corresponded to that of the control collective not exposed to DMF. CONCLUSION: In this study we tried to differentiate and quantify the interaction between DMF exposure and alcohol consumption and the influence of both substances on liver function. The experience gained from former occupational health surveillance in DMF-exposed persons and from the present study show that there are individual differences in tolerance of interactions between DMF and ethyl alcohol. Further studies are necessary for the evaluation of these individual degrees of susceptibilitiy.

L14 ANSWER 33 OF 57 MEDLINE on STN DUPLICATE 12

1999098603. PubMed ID: 9884134. Comparison of **carbohydrate-deficient transferrin**, immunoglobulin A **antibodies** reactive with acetaldehyde-modified protein and acetaldehyde-modified albumin with conventional markers of alcohol consumption. Worrall S; de Jersey J; Wilce P A; Seppa K; Hurme L; Sillanaukee P. (Department of Biochemistry, University of Queensland, Australia.) Alcoholism, clinical and experimental research, (1998 Dec) 22 (9) 1921-6. Journal code: 7707242. ISSN: 0145-6008. Pub. country: United States. Language: English.

AB **Carbohydrate-deficient transferrin** (CDT) has emerged as the best new marker for alcohol abuse. Recently plasma immunoglobulin A (IgA) reactivity with acetaldehyde (AcH)-modified proteins, or the modified proteins per se, have been proposed as a markers for high levels of alcohol consumption. In this study, we have compared CDT, IgA reactivity with AcH adducts (IgA ASR), and AcH-modified albumin with conventional markers of high alcohol intake in groups with well-defined drinking histories. The plasma activity of ALT, AST, and gamma-glutamyltransferase increased steadily with increasing alcohol consumption. CDT and AcH-modified albumin showed a similar pattern, whereas IgA ASR appeared only to be elevated after a threshold level of consumption had been reached. Neither CDT IgA ASR or AcH-modified albumin correlated strongly with any of the conventional markers or each other. This study shows that CDT, IgA ASR, AcH-modified albumin, and the conventional markers are not related, but suggests that the concurrent use of CDT and IgA ASR may lead to better identification of high alcohol intake.

L14 ANSWER 34 OF 57 MEDLINE on STN DUPLICATE 13

1998443536. PubMed ID: 9767355. **Carbohydrate-deficient transferrin** is not a useful marker for the detection of chronic

alcohol abuse. Schmitt U M; Stieber P; Jungst D; Bilzer M; Wachtler M; Heberger S; Seidel D. (Klinikum Grosshadern, Ludwig-Maximilians-University Munich, Germany.. Stieber@klch.med.uni-muenchen.de) . European journal of clinical investigation, (1998 Aug) 28 (8) 615-21. Journal code: 0245331. ISSN: 0014-2972. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: The role of **carbohydrate-deficient transferrin** (CDT) as a reliable marker for the detection of chronic alcohol abuse has been discussed controversially. METHODS: Therefore, we investigated CDT in the sera from 405 subjects with different alcohol intake. Besides healthy control subjects (n = 42), inpatients and outpatients in a department of gastroenterology (n = 325) and patients admitted to a department of otorhinolaryngology (n = 38) were studied. A total of 213 patients suffered from various forms of liver diseases, and 89 patients had liver transplantation. CDT values were determined by a double-**antibody** radioimmunoassay. RESULTS: In the 241 alcohol-abstinent subjects, CDT levels ranged from 3 to 90 units L-1 (median = 12); the 92 moderate drinkers (20-60 g of alcohol per day) showed values from 3 to 40 units L-1 (median = 12), and the 72 subjects with chronic alcohol abuse (> 60 g per day) revealed CDT levels from 3 to 100 units L-1 (median = 16). The diagnostic specificity for alcohol abuse was 86.8% for men (sensitivity 36.9%) and 95% for women (sensitivity 0%). CONCLUSION: Our data indicate that measurement of CDT does not reach clinical use in the detection of chronic alcohol abuse in an unselected population because of its insufficient specificity and sensitivity.

L14 ANSWER 35 OF 57 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 14

1999:140648 Document No.: PREV199900140648. Electrophoretic analysis on sugar chains of **carbohydrate-deficient transferrin** from patients with alcoholic liver disease. Inoue, Takahiro [Reprint author]; Ohkawa, Kiyoshi. Dep. Internal Med. I, Jikei Univ. Sch. Med., 3-25-8 Nishi-Shinbashi, Minato-Ku, Tokyo 105-0003, Japan. Japanese Journal of Electrophoresis, (Dec., 1998) Vol. 42, No. 4, pp. 239-243. print. CODEN: SBBKA4. ISSN: 0031-9082. Language: Japanese.

AB We have investigated the oligosaccharide structures of serum transferrin (Tf), and of serum **carbohydrate-deficient transferrin** (CDT) in particular, from patients with alcoholic liver disease (ALD) using lectin affinity electrophoresis coupled with **antibody**-affinity blotting and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by western blotting. By concanavalin A-affinity electrophoresis, serum CDT was separated mainly into weakly reactive and non-reactive Tfs which showed slower electrophoretic mobilities than those from the healthy controls. Moreover, nearly all of the serum CDT was non-reactive with datura stramonium agglutinin. On SDS-PAGE, the molecular masses of serum CDT were estimated to be approximately 75 and 72 kDa, which corresponded to those of partially and completely deglycosylated Tf from the healthy controls (78 kDa), respectively. These results indicated that the oligosaccharide structures of serum CDT from patients with ALD show not merely a loss of terminal sialic acids, but also the absence of asparagine-N-linked oligosaccharides. Similar result was found with serum a 1-acid glycoprotein.

L14 ANSWER 36 OF 57 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

1998:471499 Document No.: PREV199800471499. Adducts and **antibodies**: Markers of alcohol intake?. Worrall, S.; Wilee, P. A.. Univ. Queensland, Brisbane, QLD, Australia. Alcoholism Clinical and Experimental Research, (May, 1998) Vol. 22, No. 3 ABSTR. SUPPL., pp. 140A. print. Meeting Info.: Ninth Congress of the International Society for Biomedical Research on Alcoholism. Copenhagen, Denmark. June 27-July 2, 1998. International Society for Biomedical Research on Alcoholism. CODEN: ACRSDM. ISSN: 0145-6008. Language: English.

L14 ANSWER 37 OF 57 MEDLINE on STN DUPLICATE 15

1998207678. PubMed ID: 9545549. Identification of **carbohydrate deficient transferrin** forms by MALDI-TOF mass spectrometry and lectin ELISABiochim Biophys Acta 1998 Aug 24;1381(3):356. Peter J; Unverzagt C; Engel W D; Renauer D; Seidel C; Hosel W. (Institut fur Organische Chemie und Biochemie, Technische Universitat Munchen, Garching, Germany.) Biochimica et biophysica acta, (1998 Mar 12) 1380 (1) 93-101. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Transferrin was isolated from sera of patients with severe alcohol abuse and from control sera by affinity chromatography using an immobilized polyclonal **antibody** from sheep, followed by gel filtration. The purified transferrin was then separated by MonoQ chromatography. Compared to the controls, sera from heavy alcohol consumers showed two additional transferrin peaks, eluting earlier than the three main transferrin forms present in all sera. Further analysis of the isolated transferrin forms by matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) and enzyme linked immunosorbent assay with different digoxigenylated lectins (lectin ELISA) revealed that the main **carbohydrate deficient transferrin** (CDT) forms are lacking either one or both of the N-Glycan chains.

L14 ANSWER 38 OF 57 MEDLINE on STN DUPLICATE 16

2000395428. PubMed ID: 10909806. Anti-peptide **antibodies** to epitopes masked by the carbohydrate moieties in transferrin. Trimble E R; McFerran N V; Wisdom G B. (Peptide and Protein Engineering Group, School of Biology and Biochemistry, The Queen's University, Belfast, UK.) Biochemical Society transactions, (1998 Feb) 26 (1) S48. Journal code: 7506897. ISSN: 0300-5127. Pub. country: ENGLAND: United Kingdom. Language: English.

L14 ANSWER 39 OF 57 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

1998043679 EMBASE Anti-peptide **antibodies** to epitopes masked by the carbohydrate moieties in transferrin. Trimble E.R.; McFerran N.V.; Wisdom G.B.. E.R. Trimble, Peptide Protein Engineering Group, School of Biology and Biochemistry, The Queen's University, Belfast BT9 7BL, United Kingdom. Biochemical Society Transactions Vol. 26, No. 1, pp. S48 1998.
Refs: 9.
ISSN: 0300-5127. CODEN: BCSTB5
Pub. Country: United Kingdom. Language: English.

ED Entered STN: 19980227
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L14 ANSWER 40 OF 57 MEDLINE on STN DUPLICATE 17

1998006647. PubMed ID: 9347098. Is **carbohydrate-deficient transferrin** a specific marker for alcohol abuse? A study in patients with chronic viral hepatitis. Perret R; Froehlich F; Lavanchy D; Henry H; Bachman C; Pecoud A; Bianchi L; Gonvers J J. (Division of Gastroenterology, University Hospital, Lausanne, Switzerland.) Alcoholism, clinical and experimental research, (1997 Oct) 21 (7) 1337-42. Journal code: 7707242. ISSN: 0145-6008. Pub. country: United States. Language: English.

AB **Carbohydrate-deficient transferrin**, a transferrin isoform, is hailed as a new marker of chronic alcohol abuse, but its specificity is, however, not unequivocally accepted. The aim of the present study was therefore to determine **carbohydrate-deficient transferrin** levels in patients with chronic hepatitis B and C with or without documented chronic alcohol intake. **Carbohydrate-deficient transferrin** was measured using a double-**antibody** radioimmunoassay (CDTect, Pharmacia) in serum samples from 66 patients (45 males and 21 females; mean age: 39 years) with chronic viral hepatitis B (n = 20) or C (n = 46). Diagnosis of the underlying liver disease was established by liver biopsy. **Carbohydrate-deficient transferrin** levels were raised in 15 patients [23%; hepatitis B (n = 2) and hepatitis C (n = 13)].

In patients with chronic hepatitis B, the **carbohydrate-deficient transferrin** level was raised in two abstainers. In the 46 patients with chronic hepatitis C, 10 (22%) patients with an alcohol consumption of < 60 g/day for the men and 30 g/day for the women had raised **carbohydrate-deficient transferrin** levels. The overall specificity of **carbohydrate-deficient transferrin** for chronic alcohol abuse was thus 78%, suggesting an association between elevated **carbohydrate-deficient transferrin** levels and the presence of chronic viral hepatitis. **Carbohydrate-deficient transferrin** levels were not correlated with the histological grading or staging of chronic hepatitis B and C, or with biological markers of hepatic synthesis and cellular damage. Thus, an increased **carbohydrate-deficient transferrin** level may occur in patients with chronic viral hepatitis in the absence of chronic alcohol abuse. This fact should be kept in mind by physicians when using this marker to detect alcohol abuse.

L14 ANSWER 41 OF 57 MEDLINE on STN DUPLICATE 18

97462284. PubMed ID: 9316554. Efficacy of a high and accelerated dose of hepatitis B vaccine in alcoholic patients: a randomized clinical trial. Rosman A S; Basu P; Galvin K; Lieber C S. (Alcohol Research and Treatment Center, Bronx VA Medical Center, New York 10468, USA.) American journal of medicine, (1997 Sep) 103 (3) 217-22. Journal code: 0267200. ISSN: 0002-9343. Pub. country: United States. Language: English.

AB PURPOSE: A randomized, double-blind trial was conducted to compare the efficacy of a high-dose versus standard-dose hepatitis B vaccine in alcoholic patients. PATIENTS AND METHODS: One hundred ten alcoholic patients were randomized to either receive the standard dose (20 micrograms at 0.1, and 6 months) or a high dose (40 micrograms at 0, 1, 2, and 6 months) of recombinant hepatitis B vaccine (Engerix-B). Patients were monitored for relapse of drinking using self-report, serial serum **carbohydrate deficient transferrin**, and collateral verification. The final titer of **antibody** to hepatitis B surface antigen (anti-HBs) was obtained 12 months after the first vaccine dose; a seroconversion was defined as a titer greater than 10 mlU/ml. RESULTS: One hundred subjects completed the study; 10 of these had clinical or pathological evidence of cirrhosis. Thirty-six out of 48 (75%) of patients administered the high-dose regimen seroconverted compared with 24 of 52 (46%) in the standard dose group ($P < 0.005$). The mean anti-HBs titer of the high dose group was significantly greater than of the standard dose group (76.4 versus 39.4 mlU/ml, $P < 0.01$). Logistic regression demonstrated a significant effect on seroconversion for the vaccine dose ($P < 0.005$) and serum albumin ($P = 0.05$) but not for the other variables such as race, age, drinking during the study, serum creatinine, arm muscle circumference, and cirrhosis. CONCLUSIONS: A high- and accelerated-dose regimen of hepatitis B improves the serological response in alcoholic patients. This regimen (currently recommended for hemodialysis patients) should now also be considered for patients with a history of alcoholism.

L14 ANSWER 42 OF 57 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

1997:535245 Document No.: PREV199799834448. **Carbohydrate-deficient transferrin** serum values as an early marker of hepatocellular carcinoma. Stefanini, G. F. [Reprint author]; Olanda, Sandra; Foschi, F. G.; Marsigli, L.; Biselli, M.; Castelli, Elena; Capelli, M.; Lizzani, L.; Bernardi, M.; Gasbarrini, G.. Cent. Univ., Studio Trattamento Multidisciplinare, Inadeguato Alcol"G. Fontana", Ospedale S. Orsola, Bologna, Italy. Hepatology, (1997) Vol. 26, No. 4 PART 2, pp. 171A.
Meeting Info.: 48th Annual Meeting of the American Association for the Study of Liver Diseases. Chicago, Illinois, USA. November 7-11, 1997.
CODEN: HPTLD9. ISSN: 0270-9139. Language: English.

L14 ANSWER 43 OF 57 MEDLINE on STN DUPLICATE 19
97181224. PubMed ID: 9029385. Cord blood **carbohydrate-deficient transferrin** levels are markedly higher than maternal. Whitty J E; Dombrowski M P; Martier S S; Subramanian M G; Sokol R J. (Department of Obstetrics and Gynecology, Hutzel Hospital/Wayne State University, Detroit, MI 48201, USA.) Journal of maternal-fetal medicine, (1997 Jan-Feb) 6 (1) 45-8. Journal code: 9211288. ISSN: 1057-0802. Pub. country: United States. Language: English.

AB Regular, heavy alcohol intake results in transferrin that is deficient in carbohydrate moieties. **Carbohydrate-deficient transferrin** (CDT) has been used as a biologic marker of heavy alcohol exposure in nonpregnant humans. There have been no reports of CDT levels in pregnancy. Our objective was to determine maternal and cord blood levels of CDT. Parturients were recruited at delivery based on graded representative alcohol consumption, from abstainers to heavy drinkers, as determined by screeners skilled at eliciting drug and alcohol histories. Maternal and cord blood serum samples were obtained at delivery. A double **antibody** radioimmunoassay was used to determine CDT in each sample. There were 83 paired specimens analyzed by paired t tests and stepwise regression analysis. Cord blood CDT units/liter (44.0 +/- 29.5) were significantly ($P < 0.0001$) higher than maternal (18.4 +/- 7.0). Maternal and cord CDT did not correlate with race, perinatal risk score, gestational age at delivery, birth weight, Apgar scores, or reported alcohol intake. Maternal CDT levels had a significant negative correlation with cigarette smoking. Cord blood CDT levels are significantly higher than maternal. While regular, heavy alcohol consumption by adults results in serum transferrin deficient in carbohydrate moieties, the reason for elevated fetal CDT is unknown.

L14 ANSWER 44 OF 57 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
1996:841687 The Genuine Article (R) Number: VT994. Isoelectric focusing (IEF) and immunofixation for determination of disialotransferrin. Dumon M F (Reprint); Nau A; Hervouet M; Paccalin J; Clerc M. HOP ST ANDRE, CENT BIOCHIM LAB, F-33075 BORDEAUX, FRANCE (Reprint); HOP ST ANDRE, SERV MED INTERNE & THERAPEUT, F-33075 BORDEAUX, FRANCE. CLINICAL BIOCHEMISTRY (DEC 1996) Vol. 29, No. 6, pp. 549-553. ISSN: 0009-9120. Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Objectives: A new simplified method for detection and quantitation of disialontransferrin in serum is described.

Design and Methods: The method is based on polyacrylamide gel isoelectric focusing, direct immunofixation with a specific **antibody**, and measurement by computerized scanning densitometry. Disialotransferrin levels were determined in 24 teetotallers and 34 alcoholics at 3 moments during detoxification. Three groups of drinkers were arranged: group 1 (without), group 2 (with light), and group 3 (with severe hepatitis).

Results: The metho showed very good reproducibility and accuracy with a coefficient of Variation between 5 to 8%. Alcoholic patients could be clearly separated from teetotallers, with a specificity of 100% and a sensitivity of 94%. After 12 days of alcohol withdrawal, disialotransferrin Values declined in alcoholics but remained slightly high. They were not influenced by the severity of liver disease. No significant difference was found between the 3 groups.

Conclusions: An easy-to-perform, sensitive, and inexpensive method has been developed to quantify disialotransferrin that can be used by laboratories almost everywhere.

L14 ANSWER 45 OF 57 MEDLINE on STN DUPLICATE 20
97139638. PubMed ID: 8986239. Microheterogeneity with concanavalin A affinity of serum transferrin in patients with alcoholic liver disease. Inoue T; Yamauchi M; Toda G; Ohkawa K. (Tikei University School of Medicine, Tokyo, Japan.) Alcoholism, clinical and experimental research,

(1996 Dec) 20 (9 Suppl) 363A-365A. Journal code: 7707242. ISSN: 0145-6008. Pub. country: United States. Language: English.

AB Microheterogeneity of transferrin (Tf) with concanavalin A (ConA) affinity was investigated by sensitive lectin-affinity electrophoresis and **antibody**-affinity blotting technique of sera obtained from patients with alcoholic liver disease (ALD) and normal subjects. Serum Tf was separated by ConA into three bands-a strongly ConA-reactive major band (C1), a weakly reactive minor band (C2), and a non-reactive trace band (C3). The C3 fraction was significantly increased in patients with ALD before alcohol abstinence, compared with normal subjects and patients with ALD after 4 weeks of abstinence. Furthermore, a significant correlation was found between the C3 fraction and serum **carbohydrate-deficient transferrin** or gamma-glutamyl-transpeptidase. These results indicate that the microheterogeneity of serum Tf in patients with ALD may be a more complex abnormality of elongation and processing on the glycans than merely a loss of terminal sialic acids. Determination of the C3 fraction is a useful marker for ALD.

L14 ANSWER 46 OF 57 MEDLINE on STN DUPLICATE 21

95285570. PubMed ID: 7768004. **Carbohydrate-deficient transferrin** and false-positive results for alcohol abuse in primary biliary cirrhosis: differential diagnosis by detection of mitochondrial autoantibodies. Bean P; Sutphin M S; Liu Y; Anton R; Reynolds T B; Shoenfeld Y; Peter J B. (Specialty Laboratories, Inc., Santa Monica, CA 90404-3900, USA.) Clinical chemistry, (1995 Jun) 41 (6 Pt 1) 858-61. Journal code: 9421549. ISSN: 0009-9147. Pub. country: United States. Language: English.

AB Primary biliary cirrhosis (PBC) is one of the few nonalcohol-induced liver pathologies that causes false positives in assays of **carbohydrate-deficient transferrin** (CDT) for diagnosing alcohol abuse. CDT was quantified by isoelectric focusing-immunoblotting-laser densitometry (IEF-IB-LD) analysis of serum from 117 women: 57 PBC patients, 20 alcohol abusers, and 40 healthy donors. Only 5% (3 of 57) of PBC patients were positive at the densitometric cutoff value chosen (> 90% specificity). Serum samples from 15 PBC patients were further evaluated by IEF-IB-LD and CDTect chromatography-RIA. Receiver-operating characteristic (ROC) analysis showed that IEF-IB-LD better discriminated between PBC and alcohol abuse than CDTect did. By ROC analysis, mitochondrial autoantibodies to pyruvate dehydrogenase antigen M2 detected by enzyme immunoassay yielded optimal test performance for diagnosing PBC. Of six patients falsely positive for CDT by CDTect, five (83%) tested M2-positive. Thus, abnormal CDT results should be further evaluated by mitochondrial **antibody** testing in patients with findings compatible with PBC.

L14 ANSWER 47 OF 57 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

1995:214922 The Genuine Article (R) Number: QN286. **CARBOHYDRATE-DEFICIENT TRANSFERRIN** IN ALCOHOLICS WITH LIVER-DISEASE. CALDWELL S H (Reprint); HALLIDAY J W; FLETCHER L M; KULAGA M; MURPHY T L; LI X M; DICKSON R C; KIYASU P K; FEATHERSTON P L; SOSNOWSKI K. UNIV VIRGINIA, HLTH SCI CTR, DEPT INTERNAL MED, DIV GASTROENTEROL, BOX 145, CHARLOTTESVILLE, VA 22908 (Reprint); SALEM VET ADM MED CTR, SALEM, VA; UNIV QUEENSLAND, JOINT LIVER PROGRAMME, QUEENSLAND INST MED RES, BRISBANE, QLD, AUSTRALIA; UNIV MIAMI, CTR LIVER DIS, MIAMI, FL; VET ADM MED CTR, MIAMI, FL. JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY (MAR-APR 1995) Vol. 10, No. 2, pp. 174-178. ISSN: 0815-9319. Publisher: BLACKWELL SCIENCE PUBL AUSTR, 54 UNIVERSITY ST, P O BOX 378, CARLTON 3053, AUSTRALIA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB To assess the relationship between **carbohydrate-deficient transferrin** (CDT) and alcoholic liver disease, we measured the ratio of **carbohydrate-deficient transferrin** to total transferrin (rCDT) in 32 male alcoholics with liver disease (Child-Pugh class A, 8; B, 11; C, 13) and 14 male alcoholics

without clinically evident liver disease. Twenty of 32 with liver disease and six of 14 without clinically apparent liver disease had recent abstinence. The 32 patients with liver disease were assessed, in addition to the Child-Pugh class, using a linear prognostic score, the Combined Clinical and Laboratory Index (CCLI). Transferrin and CDT were measured by isocratic anion exchange chromatography and a radio-immunoassay. When the total group (n = 46) was divided into those with recent abstinence (n = 26) and those without (n = 20), the rCDT was lower in the abstainers than non-abstainers (0.7 +/- 0.6 vs 2.9 +/- 2.4, P < 0.005). Similarly, abstainers with liver disease (n = 20) had a significantly lower rCDT than non-abstainers (n = 12) with liver disease (0.7 +/- 0.7 vs 3.5 +/- 2.8, P < 0.005). The rCDT in the 20 abstaining patients with liver disease did not differ significantly between Child-Pugh classes. Furthermore, there was no correlation between the CCLI and rCDT (r = 0.05). We conclude that the relationship between rCDT and alcohol abuse is not appreciably altered by the presence of clinically severe liver disease in male alcoholics.

L14 ANSWER 48 OF 57 MEDLINE on STN DUPLICATE 22
95150262. PubMed ID: 7847598. Comparison of different methods for detecting **carbohydrate-deficient transferrin**
. Sillanaukee P; Lof K; Harlin A; Martensson O; Brandt R; Seppa K. (Biomedical Research Center, Alko Ltd., Helsinki, Finland.) Alcoholism, clinical and experimental research, (1994 Oct) 18 (5) 1150-5. Journal code: 7707242. ISSN: 0145-6008. Pub. country: United States. Language: English.

AB Different methods for detecting **carbohydrate-deficient transferrin** (CDT) were compared. In addition, their efficiency for detecting alcohol abuse among men not having clinical evidence of liver disease was studied in controls (n = 26), weekend (n = 16) and daily (n = 12) heavy drinkers, and alcoholics (n = 28). Comparisons were made between anion-exchange separation of iron-saturated transferrin (Tf) by microcolumns (CDTect) and by the Fast Protein Liquid Chromatography (FPLC% and FPLC-MG), followed by double-**antibody** radioimmunoassay of collected fractions. Tf fractions with pl > or = 5.7 were also measured by two different isoelectric focusing (IEF) methods, followed by immunofixation (SA-IEF-CDT and IEF-CDT-TOT), the latter method being used also for detection of asialotransferrin (IEF-CDT-AS). The cut-off was 20 units/liter for CDTect, 4.4% of total Tf for SA-IEF-CDT, and the mean +2 sd of the control group for FPLC-MG (as mg/liter of Tf), FPLC-%, IEF-CDT-TOT, and IEF-CDT-AS (all as percentage of Tf). The overall accuracies (combining sensitivity and specificity) for detecting heavy drinkers of CDTect, FPLO (mg/liter), FPLC (%), SA-IEF-CDT, IEF-CDT-TOT, and IEF-CDT-AS were 63%, 59%, 61%, 74%, 57%, and 63%, respectively; for detecting alcoholics, 87%, 83%, 81%, 89%, 37%, and 76%, respectively. In conclusion, the methods were in rather good agreement with each other. Diagnostic characteristics among heavy drinkers and correlations between methods differed slightly, probably depending on the ability of different methods to separate and detect asialo-, monosialo-, and disialotransferrin.(ABSTRACT TRUNCATED AT 250 WORDS)

L14 ANSWER 49 OF 57 EMBASE on STN DUPLICATE 23
94232282 EMBASE Document No.: 1994232282. [Laboratory diagnostic and control of alcohol abuse - By **carbohydrate-deficient-transferrin** (CDT)]. LABORDIAGNOSTIK UND KONTROLLE DES ALKOHOLABUSUS - EIN PLADOYER FUR **CARBOHYDRATE-DEFICIENT-TRANSFERRIN** (CDT). Arndt T.; Gressner A.M.; Kropf J.. Klinikum, Philipps-Universitat, Abteilung fur Klinische Chemie, Baldingerstrasse,D-35033 Marburg, Germany. Medizinische Welt Vol. 45, No. 6, pp. 247-257 1994.
ISSN: 0025-8512. CODEN: MEWEAC
Pub. Country: Germany. Language: German. Summary Language: German; English.

ED Entered STN: 940831

AB Among the common available markers of alcohol abuse (γ-GT, AST,

AST/ALT-ratio), serum-CDT-concentration proves to be superior, especially with regard to specificity. Increased serum-CDT is typical for patients with alcohol abuse (>50-80 g/d for at least 7 consecutive days) or alcohol-induced liver diseases. Therefore, serum-CDT is useful for detection and control of alcohol abuse as well as for differentiation of alcohol-related and non-alcoholic diseases. Potential markers of alcohol abuse like acetaldehyde-amine-adducts and **antibodies** against them, apolipoproteins AI and AII, mitochondrial AST, 5-hydroxytryptophol require further clinical and analytical evaluation. The same applies to potential markers of genetically predisposition to alcohol abuse like event-related potential P300, monoamine oxidase activity in platelets and adenylate cyclase activity in platelets and thrombocytes.

L14 ANSWER 50 OF 57 MEDLINE on STN DUPLICATE 24
94324715. PubMed ID: 8048718. **Carbohydrate-deficient transferrin** during 3 weeks' heavy alcohol consumption. Salmela K S; Laitinen K; Nystrom M; Salaspuro M. (Research Unit of Alcohol Diseases, University of Helsinki, Finland.) Alcoholism, clinical and experimental research, (1994 Apr) 18 (2) 228-30. Journal code: 7707242. ISSN: 0145-6008. Pub. country: United States. Language: English.

AB To study the effect of controlled heavy drinking of 60 g ethanol/day for 3 weeks on **carbohydrate-deficient transferrin** (CDT), a commercial double **antibody** kit (CDTect) was used. By the end of the third drinking week, a statistically significant increase in the mean CDT level was observed. When compared to AST and gamma-glutamyltransferase, CDT was a more informative marker. However, only in 2 of the 10 volunteers did CDT exceed the upper normal level (20 units/liter) recommended by the manufacturer. This indicates that the sensitivity of CDT to detect heavy drinking is lower than that previously reported. The higher accuracy has in general been obtained in studies comparing healthy controls with a low alcohol consumption to alcoholics with an alcohol consumption higher than that used in the present experiment. Our results suggest that it remains to be established whether CDT, although better than AST and gamma-glutamyltransferase, will provide a clinically useful tool in identifying heavy drinkers in populations covering a wide range of alcohol consumption.

L14 ANSWER 51 OF 57 MEDLINE on STN DUPLICATE 25
95008264. PubMed ID: 7923813. Enzymes and circulating proteins as markers of alcohol abuse. Goldberg D M; Kapur B M. (Department of Clinical Biochemistry, University of Toronto, Ontario, Canada.) Clinica chimica acta; international journal of clinical chemistry, (1994 May) 226 (2) 191-209. Ref: 98. Journal code: 1302422. ISSN: 0009-8981. Pub. country: Netherlands. Language: English.

AB The identification of alcohol abuse is an important social and clinical objective for which various biochemical procedures have been utilized, serum enzymes and circulating proteins being predominant. Tests are required to detect alcohol abuse as screening procedures in the general population as well as for the specific diagnosis of those presenting as hospital inpatients or outpatients, especially when liver disease is present or suspected. The amino-transferases are of limited value, although the mitochondrial isoenzyme of aspartate amino-transferase has been strongly advocated and is quite useful in detecting alcoholics among patients with liver disease. Gammaglutamyl transferase, by contrast, is raised in all forms of liver disease but can identify 30-50% of those consuming excessive amounts of alcohol before organic damage becomes manifest. Serum **carbohydrate-deficient transferrin** (CDT) is raised in many alcohol abusers without and most with liver damage, but is rarely elevated in other forms of liver disease. Haemoglobin-associated acetaldehyde, the newest biochemical index to be evaluated in alcoholics, is one of several adducts formed by the reaction of acetaldehyde with various proteins, and **antibodies** to these adducts may contribute, at least in part, to immunological tissue damage provoked by chronic excessive consumption of alcohol. Its assay is technically complex and it appears to be present in higher concentrations

in heavy drinkers than in those who fulfill the criteria of addictive alcohol abuse. Many other markers have been introduced in the last decade but the search for a reliable index continues. CDT comes closest at the present time to matching the desired specificity, although it is of limited value in screening unselected non-hospitalized subjects.

L14 ANSWER 52 OF 57 CAPLUS COPYRIGHT 2005 ACS on STN
1995:327214 Document No. 122:100846 Abnormal hemoglobin and **carbohydrate-deficient transferrin** demonstrated by mass spectrometry. Nakanishi, Toyofumi (Dep. Clin. Pathol., Osaka Med. Coll., Japan). Osaka Ika Daigaku Zasshi, 53(2), 64-71 (Japanese) 1994. CODEN: OIDZAU. ISSN: 0030-6118. Publisher: Osaka Ika Daigaku Igakkai.

AB Transferrin and hemoglobin were analyzed by new techniques of mass spectrometry. Serum transferrin precipitated with anti-transferrin serum was analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALD/TOF-MS). The transferrin-**antibody** complex in the immunoppts. was separated into transferrin and IgG in an acidic pH, which is the usual condition of loading on MALD/TOF-MS. Ions of IgG and other minor components were not superimposed on the transferrin ions. Transferrin isoforms with different carbohydrate contents could be identified by this simple method more easily than by affinity chromatog., which requires the time-consuming preparation of an insolubilized specific **antibody.** Transferrin isoform, with a mol. weight ca. 2.2 kDa less than that of normal transferrin, which is present in the serum of patients with carbohydrate deficient glycoprotein (CDG) syndrome, was identified by this method. Electrospray ionization mass spectrometry (ESI-MS) showed that the abnormal α-chain of a Hb variant, HbM-Osaka (Boston) had a mol. weight between that of histidine and tyrosine. The exact weight was also detected by MALD/TOF-MS. The peak of the abnormal subunit was identified in the spectrum of whole globin prepared from the hemolyzate of a patient. These techniques can provide a routine clin. tool for the diagnosis of inborn errors of protein structure.

L14 ANSWER 53 OF 57 MEDLINE on STN DUPLICATE 26
94084919. PubMed ID: 8261626. Semi-automatic method for determination of different isoforms of **carbohydrate-deficient transferrin.** Lof K; Koivula T; Seppa K; Fukunaga T; Sillanaukee P. (Biomedical Research Center, Alko Ltd, Department of Clinical Chemistry.) Clinica chimica acta; international journal of clinical chemistry, (1993 Aug 31) 217 (2) 175-86. Journal code: 1302422. ISSN: 0009-8981. Pub. country: Netherlands. Language: English.

AB **Carbohydrate deficient transferrin** (CDT) has been reported to be one of the best biochemical markers of alcohol abuse. However, a need still exists for a simple and practical method for widespread laboratory use. A semi-automatic (SA) isoelectric focusing (IEF) assay for CDT (SA-IEF-CDT) by a Phast System is introduced here. Different isoforms of transferrin were separated by IEF on polyacrylamide gels (pI 4.0-6.5) and located by immunofixation with an anti-transferrin serum. The precipitation bands were stained with Coomassie Brilliant Blue and quantitated densitometrically. The present method gave a picture of the relative amounts of 10 different transferrin isoforms. The percentage of CDT with pI > or = 5.7 (representing di-, mono- and asialotransferrin) was calculated. For comparisons transferrin bands with pI > or = 5.6 (tri-, di-, mono-, and asialotransferrin), pI > or = 5.8 (mono- and asialotransferrin) and pI > or = 5.9 (asialotransferrin) as well as GGT, ASAT and ALAT were calculated. The method showed good linearity and it identified different isoforms in concentrations of < 10 mg/l of transferrin. The correlation of the present method with a commercially available method employing anion exchange followed by double **antibody** RIA (AE-RIA-CDT) was good (n = 38, r = 0.924). In 19/20 (95%) of healthy controls, the CDT value was below 4.4% (mean + 2 S.D.) of total transferrin, while higher values were observed in all 20 (100%) alcoholics. In conclusion, the developed semi-automatic method is a practical and reliable alternative for determination of different

transferrin isoforms.

L14 ANSWER 54 OF 57 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

1992:301551 The Genuine Article (R) Number: HQ893. BIOLOGIC TESTS FOR DETECTION OF ALCOHOL-ABUSE. RENNER E L (Reprint). UNIV BERN, INST KLIN PHARMAKOL, MURTENSTR 35, CH-3010 BERN, SWITZERLAND (Reprint). SCHWEIZERISCHE MEDIZINISCHE WOCHENSCHRIFT (25 APR 1992) Vol. 122, No. 17, pp. 614-618. ISSN: 0036-7672. Publisher: SCHWABE & CO AG VERLAG, FARNSBURGERSTRASSE 8, CH-4132 MUTTENZ 1, SWITZERLAND. Language: German.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Since most patients with an alcohol problem downplay their alcohol consumption, reliable tests for detection of alcohol abuse would be of value in clinical practice. Single determinations of common laboratory tests such as gamma-glutamyl transpeptidase. transaminases or mean corpuscular volume are only of limited reliability in detecting alcohol abuse. Most newer parameters, including the serum ASAT/ALAT ratio, the ratio of mitochondrial to total ASAT in serum, and serum levels of acetaldehyde-hemoglobin adducts or of **antibodies** against acetaldehyde adducts, still do not allow us to discriminate reliably enough between alcoholic and nonalcoholic liver diseases. Beta-hexosaminidase activity and desialylated transferrin levels in serum appear to be the most promising tests for detecting alcohol abuse in the future. They require, however, additional validation and technical simplification respectively before they are suitable for daily clinical use. Thus, it remains valid that no single test can replace a careful history, clinical examination and laboratory results in detecting patients with alcohol problems.

L14 ANSWER 55 OF 57 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

1991:597357 The Genuine Article (R) Number: GL861. EFFECT OF ACETALDEHYDE ON HEMOGLOBIN - HBA1ACH AS A POTENTIAL MARKER OF HEAVY DRINKING. SILLANAUKEE P (Reprint); SEPPA K; KOIVULA T. TAMPERE UNIV, CENT HOSP, DEPT CLIN CHEM, SF-33520 TAMPERE, FINLAND (Reprint); UNIV TAMPERE, DEPT PUBL HLTH, SF-33520 TAMPERE, FINLAND. ALCOHOL (SEP-OCT 1991) Vol. 8, No. 5, pp. 377-381. ISSN: 0741-8329. Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The appearance of a new acetaldehyde-induced hemoglobin fraction, HbA1ach, and the effect of alcohol consumption on it and on the ratio of HbA1ach and glycated hemoglobin, HbA1c, were studied in vivo by cation exchange liquid chromatography. The mean +/- SEM of blood HbA1ach level was 171 +/- 13.10(-3%) of total hemoglobin as measured in 34 male teetotallers. Blood HbA1ach levels of 127 social drinkers (182 +/- 6.10(-3%) were compared with those of 72 heavy drinkers (213 +/- 8.10(-3%), $p < 0.01$), 79 alcoholics (209 +/- 6.10(-3%), $p < 0.01$) and 16 diabetics (419 +/- 28.10(-3%), $p < 0.001$). HbA1ach correlated positively with HbA1c ($p < 0.001$) and negatively with HbA(o) ($p < 0.001$). The ratio of HbA1ach/HbA1c was effective in detecting the alcohol-induced increase in the HbA1ach fraction because the ratio reduced the disturbing effect of glucose. The sensitivity of the HbA1ach/HbA1c ratio was 33% in the heavy drinker group as compared to 40% of gamma-glutamyltransferase and 24% of mean corpuscular volume. The HbA1ach fraction and the HbA1ach/HbA1c ratio seem to be valuable in detecting excessive alcohol consumption in its early phase.

L14 ANSWER 56 OF 57 MEDLINE on STN DUPLICATE 27

91144829. PubMed ID: 2288734. [Diagnosis of alcoholism based on detection of a transferrin variant by polyacrylamide gel electrophoresis and immunoblotting]. Die Diagnose von Alkoholismus auf der Grundlage des Nachweises einer Transferrinvariante durch Polyacrylamid-Gelelektrophorese und Immunoblotting. Reisinger P W; Soyka M. (Anatomischen Anstalt, Lehrstuhl II, Ludwig-Maximilians-Universitat Munchen.) Blutalkohol, (1990

Nov) 27 (6) 427-33. Journal code: 0372531. ISSN: 0006-5250. Pub. country: GERMANY: Germany, Federal Republic of. Language: German.

AB A sensitive method is discribed for the diagnosis of alcoholism. The method is based on the detection of a transferrin variant (**carbohydrate deficient transferrin** = CDT) in plasma of alcoholics. The determination of CDT, the presence of which is characteristic for chronic alcoholism, is performed in three steps: The plasma proteins are separated by polyacrylamide gel electrophoresis and then transferred electrophoretically onto a nitrocellulose sheet; finally, the transferrin types on the nitrocellulose sheet are specifically detected by an **antibody** reaction. With the exception of certain cases (genetic variants, rare diseases) CDT is found only during chronic alcohol consumption. In comparison to other markers for chronic alcoholism an advantage of CDT is its higher specificity. A further advantage of the method is that CDT can be identified with high sensitivity by the use of a relatively small amount of technical equipment.

L14 ANSWER 57 OF 57 MEDLINE on STN DUPLICATE 28

87097874. PubMed ID: 3099592. Micro anion exchange chromatography of **carbohydrate-deficient transferrin** in serum in relation to alcohol consumption (Swedish Patent 8400587-5). Stibler H; Borg S; Joustra M. Alcoholism, clinical and experimental research, (1986 Oct) 10 (5) 535-44. Journal code: 7707242. ISSN: 0145-6008. Pub. country: United States. Language: English.

AB A new simplified and rapid method for detection and quantitation of " **carbohydrate-deficient transferrin**" in serum is described. The method is based on isocratic anion exchange chromatography of isotransferrins in disposable microcolumns followed by a double **antibody** transferrin radioimmune assay. This technique, which separates all transferrin components isoelectric above pH 5.65, showed a very good reproducibility and accuracy with a coefficient of variation between 5 and 9%. 77 alcoholic patients could be clearly separated from 80 healthy "normal consumers" and 33 total abstainers with a specificity of 100% and a sensitivity of 91%. The values were significantly correlated to the amount of alcohol consumed during the latest month, and declined in abstaining alcoholics with a mean biological half-life of 17 days. Elevated levels occasionally appeared in healthy individuals after daily consumption of 60 g of ethanol during a 10-day period. In a sample of 187 patients with nonalcohol-related conditions only 2% false-positive values were found. This method is suggested as a potential tool for detecting and monitoring alcohol abuse.

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=> s (althaus h?/au)
L15         387 (ALTHAUS H?/AU)

=> s l15 and antibody
L16          30 L15 AND ANTIBODY

=> s l16 and carbohydrate deficient transferrin
L17           4 L16 AND CARBOHYDRATE DEFICIENT TRANSFERRIN

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PROCESSING COMPLETED FOR L17
L18            3 DUP REMOVE L17 (1 DUPLICATE REMOVED)

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L18 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN

2004:17426 Document No. 140:56037 Anti-**carbohydrate deficient transferrin** (CDT) specific **antibody** and method for its production. **Althaus, Harald** (Dade Behring Marburg G.m.b.H., Germany). Eur. Pat. Appl. EP 1378521 A1 20040107, 21 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI,

LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK. (German). CODEN: EPXXDW. APPLICATION: EP 2003-11334 20030519. PRIORITY: DE 2002-10230550 20020705.

AB The invention concerns **antibodies** to **carbohydrate deficient transferrin** (CDT) that bind to the following sequences in CDT: (1) WARSMGGKEDLIWELL ; (2) TTEDSIAKIMNGEADAMSLDGGF; (3) SKLSMGSGLNLSEPN; (4) YEKYLGEEYVKAV. The **antibodies** bind to CDT in aqueous solns. without a solid phase. For the production of monoclonal **antibodies** animals are immunized with non-glycosylated transferrin; spleen cells of the animals are fused with myeloma cells, thus **antibody**-producing hybrid cells are produced. The **antibodies** can be used for serodiagnosis of alc. patients.

L18 ANSWER 2 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2005:320560 Document No.: PREV200510106919. A novel particle-enhanced assay for the immuno-nephelometric determination of **carbohydrate-deficient transferrin.** Kraul, D. [Reprint Author]; Hackler, R.; **Althaus, H.**. Univ Marburg, Klin Innere Med Kardiol, AG Pravent Kardiol, D-35032 Marburg, Germany. Alcoholism Clinical and Experimental Research, (AUG 2004) Vol. 28, No. 8, Suppl. S, pp. 34A. Meeting Info.: 12th International Congress of the International-Society-for-Biomedical-Research-on-Alcoholism. Heidelberg, GERMANY. September 29 -October 02, 2004. Int Soc Biomed Res Alcoholism. CODEN: ACRSDM. ISSN: 0145-6008. Language: English.

L18 ANSWER 3 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 1

2003:361856 Document No.: PREV200300361856. Development and evaluation of a new **carbohydrate-deficient transferrin** (CDT)-specific monoclonal **antibody. Althaus, H.** [Reprint Author]; Hackler, R.; Fischer, B. [Reprint Author]; Schaefer, J. R.; Walter, G. [Reprint Author]; Harthus, H. P. [Reprint Author]. Dade Behring Marburg GmbH, Marburg, Germany. Clinical Chemistry, (June 2003) Vol. 49, No. S6, pp. A113. print. Meeting Info.: 55th Annual Meeting of the AACC (American Association for Clinical Chemistry). Philadelphia, PA, USA. July 20-24, 2003. American Association for Clinical Chemistry. CODEN: CLCHAU. ISSN: 0009-9147. Language: English.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	191.43	191.64

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Takahiro Inoue[1]
Masayoshi Yamauchi[1]
Kiyoshi Ohkawa[2]

[1]Department of Internal Medicine (I)
[2]Department of Biochemistry (I), The Jikei University School of Medicine, Tokyo, Japan

Structural studies on sugar chains of carbohydrate-deficient transferrin from patients with alcoholic liver disease using lectin affinity electrophoresis

It is well-known that microheterogeneity of human serum transferrin observed in alcoholics manifests as sialic acid-deficient transferrin isoforms, otherwise known as carbohydrate-deficient transferrin (CDT). A recent study demonstrated that serum CDT lacked one or both of the entire carbohydrate chains but the investigation required several troublesome procedures. The aim of the present study was to confirm the sugar chain structures of serum transferrin, and of serum CDT in particular, from patients with alcoholic liver disease (ALD) using conventional lectin affinity electrophoresis which might be useful in the clinical setting. The serum CDT obtained from ALD-patients was partially purified using an anion exchanger. Serum transferrin and the partially purified serum CDT were investigated by concanavalin A (Con A)- and *Datura stramonium* agglutinin (DSA)-affinity electrophoresis followed by antibody-affinity blotting and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Western blotting. By Con A-affinity electrophoresis, serum CDT was separated into weakly reactive and nonreactive transferrins which showed slower electrophoretic mobilities than those from the healthy controls. Moreover, nearly all of the serum CDT was nonreactive with DSA. On SDS-PAGE, the molecular masses of serum CDT were estimated to be approximately 75 and 72 kDa, which corresponded to those of partially and completely deglycosylated transferrin obtained from the healthy controls (78 kDa), respectively. In conclusion, these results indicated that the sugar chain structures of serum CDT from patients with ALD show not merely a loss of terminal sialic acids, but also the absence of asparagine-*N*-linked oligosaccharides.

Keywords: Carbohydrate-deficient transferrin / Alcoholic liver disease / Lectin affinity electrophoresis / Sodium dodecyl sulfate-polyacrylamide gel electrophoresis EL 3308

1 Introduction

Serum transferrin associated with alcohol abuse is now known to consist of sialic acid-deficient transferrin isoforms with isoelectric points (p*I* values) $\geq$ 5.7, which are otherwise known as carbohydrate-deficient transferrin (CDT) [1–5]. We previously reported that concanavalin A (Con A)-nonreactive transferrin was significantly increased in patients showing high levels of serum CDT with alcoholic liver disease (ALD) [6]. Con A has an affinity for biantenneary complex-type oligosaccharides that link to most normal serum transferrin, but lacks an affinity for both highly branched complex-type oligosaccharides, such as those with a tri- and/or tetraantennary structure and bisecting-glucosaminylated oligosaccharides [7–9]. Accordingly, our previous results suggested that the contents of carbohydrate residues in serum transferrin increased in ALD patients. However, Stibler and Berg [10] reported that the concentrations of sialic acid, galactose and *N*-acetylglucosamine of purified transferrin were reduced in alcoholic patients as compared with healthy controls. These findings suggested that serum transferrin in ALD might give rise to more complex microheterogeneity than the mere loss of terminal sialic acids. Furthermore, serum CDT has been reported recently to lack one or both of the entire carbohydrate chains [11]. To clarify these findings in the present study, the sugar chain structures of serum transferrin, and of serum CDT in particular, from patients with ALD were investigated by a highly sensitive and clinically applicable lectin-affinity electrophoresis coupled with antibody-affinity blotting and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting analysis.

Correspondence: Takahiro Inoue, M.D., Department of Internal Medicine (I), The Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105-0003, Japan
E-mail: ramses@mxr.meshnet.or.jp
Fax: +81-3-3435-0569

Abbreviations: **ALD**, alcoholic liver disease; **CDT**, carbohydrate-deficient transferrin; **Con A**, concanavalin A; **DSA**, *Datura stramonium* agglutinin; **HRP**, horseradish peroxidase

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2 Materials and methods

2.1 Patients

Serum samples were obtained from 16 patients with ALD before and after four weeks of alcohol abstinence, four patients with ALD before abstinence, and seven non-alcoholic healthy controls. The samples were stored at –40°C until use. ALD was diagnosed on the basis of the histological criteria proposed by the Japanese Research Group for Alcoholic Liver Disease [12]. All patients were negative for HBs antigen and anti-HCV antibody. ALD patients had received one of three diagnoses: there were six cases of alcoholic fatty liver, 11 cases of alcoholic hepatic fibrosis, and three cases of alcoholic liver cirrhosis. Diagnosis was established by echo-guided liver biopsy. The serum levels of aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase and CDT were significantly higher in all ALD patients than in the healthy controls.

2.2 Assay of serum transferrin

Human serum transferrin was assayed using the sandwich enzyme-linked immunosorbent assay method reported previously [13]. This assay system is not affected by the extent of desialylation of serum transferrin.

2.3 Partial purification of serum CDT

Serum CDT was collected by the Axis %CDTri TIA kit (Axis Biochemicals ASA, Oslo, Norway). In brief, the iron-saturated transferrin sample was applied to an anion exchange microcolumn retaining serum transferrin with a high sialic content. The flow-through fraction contained only serum CDT of total serum transferrin and was used as a serum CDT sample. The fraction bound to the column was eluted with Bis-Tris buffer, pH 6.15, 0.5 M NaCl, 0.02% sodium azide and was used as a CDT-free serum. All steps were performed at room temperature. The manufacturer's instructions for this kit showed that the column could not retain the trisialo transferrin as well as the asialo, nonosialo, and disialo transferrin.

2.4 Desialylation of serum transferrin or CDT

Desialylated transferrin was prepared by extensive digestion of sera or partially purified serum CDT with neuraminidase from *Clostridium perfringens* (Nakarai Chemical, Chiba, Japan). Sera diluted 1:240 and CDT samples diluted 1:5 with 10 mM Na-phosphate, pH 7.2, to give a final transferrin concentration of approximately 10–40 mg/mL, were incubated with 1 U/mL neuraminidase for 16 h at 37°C.

2.5 Deglycosylation of serum transferrin

Completely deglycosylated transferrin was prepared as follows. Sera from healthy controls were diluted 1:240 with 0.2 M Na-phosphate, pH 7.2, containing 1% 2-mercaptoethanol, then incubated with 2 U of *N*-glycosidase F (Sigma, St. Louis, MO, USA) for 24 h at 37°C [14]. Partially deglycosylated transferrin was prepared under the same conditions as described above except for the omission of 2-mercaptoethanol.

2.6 Lectin affinity electrophoresis and antibody-affinity blotting

Lectin affinity electrophoresis and antibody-affinity blotting analyses of transferrin were performed according to a previously reported method [13, 15]. Briefly, antibody-coated nitrocellulose membranes were prepared by incubation with 100 mg/mL affinity-purified goat antibody to human transferrin, then fixation with glutaraldehyde vapor and neutralization with $NaBH_4$. After washing with 20 mM Tris-HCl, pH 7.5, 0.5 M NaCl (TBS), the nitrocellulose membrane was blocked with 0.5% Tween 20 in TBS. Lectin affinity electrophoresis was performed on 1.2% agarose gel plates (Litex type HSA; Paesel, Frankfurt, Germany) in a veronal buffer (pH 8.6, ionic strength 0.025) containing either 1 mg/mL Con A (Hohnen Oil, Tokyo, Japan) or 0.4 mg/mL *Datura stramonium* agglutinin (DSA; E–Y Laboratories, San Mateo, CA, USA). As sialylation of the terminal galactose residues abolished the affinity of highly branched complex-type oligosaccharides for DSA [16, 17], neuraminidase-treated samples were subjected to DSA-affinity electrophoresis. Two mL of sera diluted 1:240 with veronal buffer or CDT samples dialyzed against veronal buffer were electrophoresed on the plates at 10°C until bromophenol blue migrated 10 cm from the origin. Separated transferrin bands were transferred by capillary blotting to antibody-coated nitrocellulose membranes. The nitrocellulose membranes were then washed in 0.05% Tween 20 in TBS, incubated with 500-fold diluted rabbit anti-human transferrin IgG (Dakopatts, Glostrup, Denmark), followed by treatment with 1000-fold diluted swine antibody to rabbit IgG labeled with horseradish peroxidase (HRP; Dakopatts). Color was developed by treatment with 3,3′-diaminobenzidine tetrachloride (Polyscience, Warrington, PA, USA) and H_2O_2 in TBS. The color-developed papers were washed, dried, and scanned with a densitometer using NIH image software. In order to exclude the effect of varying proportions of iron saturation on electrophoretic mobilities, sera were diluted to a high degree with veronal buffer (pH 8.6), then incubated for over 2 h at 4°C and CDT samples were dialyzed overnight against veronal buffer at 4°C. Electrophoretic mobilities of transferrin

bands in lectin affinity electrophoresis were not affected by the extent of iron saturation (data not shown).

2.7 SDS-PAGE and Western blot analysis

Serum samples containing CDT (20–80 ng of transferrin) were subjected to SDS-PAGE under reducing conditions using 7.5% homogeneous gel [18]. Separated proteins were electrotransferred to a nitrocellulose membrane with the semidry method [19]. After blocking, a membrane was incubated with anti-human transferrin antibody followed by HRP-labeled second antibody. The bands were visualized by the method described in Section 2.6.

2.8 Statistical analysis

All data were expressed as mean ± standard error (S.E.). Statistical analysis was performed by Student t-test. The Spearman correlation coefficient (r) was used for correlation analysis. A P value of 0.05 was considered to be significant.

3 Results

3.1 Lectin affinity electrophoresis

Using Con A-affinity electrophoresis as shown in Fig. 1, serum transferrin from healthy controls was separated into three bands – a nonreactive trace band (C1), a weakly reactive minor band (C2), and a strongly reactive major band (C3). On the other hand, Con A separated serum transferrin from ALD patients with high serum CDT levels into five bands including three bands corresponding to C1, C2 and C3 observed in the healthy controls. The remaining two bands, which were named C1′ and C2′, migrated between C1 and C2 and between C2 and C3, respectively. Moreover, both C1′ and C2′ disappeared after four weeks of alcohol abstinence. In order to clarify the properties of these additional bands observed in ALD patients, partially purified CDT from ALD patients was also analyzed by Con A-affinity electrophoresis. Serum CDT was resolved into three bands corresponding to C1′, C2′, and a trace of C3. On Con A-affinity electrophoresis, the treatment of sera from healthy controls with neuraminidase diminished the electrophoretic mobilities of C1, C2, and C3 because of the removal of sialic acids, which were the only negatively charged carbohydrate residues in serum transferrin (Fig. 2). Neuraminidase treatment of serum CDT reduced the electrophoretic mobility of C2′, but did not affect the mobility of C1′. Furthermore, the electrophoretic mobility of neuraminidase-treated (asialo-) C1, C2 and C3 did not correspond to that of C1′. In order to evaluate the effect of the affinity of C1′ and C2′ for Con A on their respective electrophoretic mobilities, serum

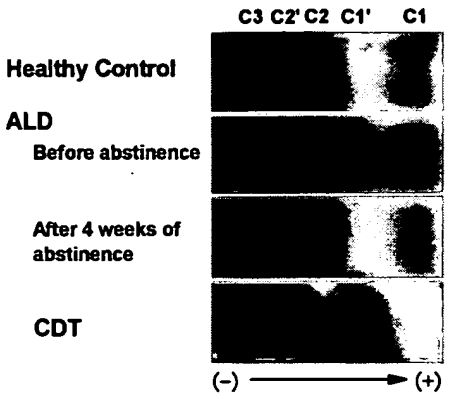

Figure 1. Con A-affinity electrophoretic patterns of serum transferrin from a healthy control and an ALD patient (before abstinence, after four weeks of abstinence and partially purified serum CDT); (–), denotes origin.

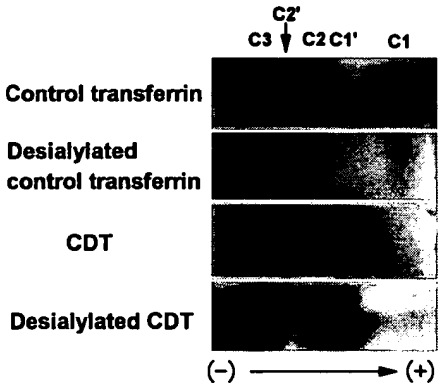

Figure 2. Electrophoretic profiles of serum transferrin separted by Con A-affinity electrophoresis after neuraminidase treatment.

CDT was subjected to agarose gel electrophoresis without lectin. Most of the serum CDT was separated into two bands (called F1 and F2, in order of decreasing mobility), which showed slight reductions in electrophoretic mobility because of high pI values, as compared with that of serum transferrin from the healthy controls. Neuraminidase treatment of serum CDT reduced the mobility of F1 but did not affect the mobility of F2. Moreover, a comparison of the electrophoretic mobilities of serum CDT bands separated by Con A-affinity electrophoresis with those by lectin-free agarose gel electrophoresis revealed that C1′ was similar to F2 (data not shown).

DSA-affinity electrophoresis was carried out to determine whether the appearance of C1′ and C2′ in ALD patients was due to the presence of highly branched complex-type oligosaccharides with tri- and/or tetraantennary structures or other abnormal oligosaccharides. As shown in Fig. 3,

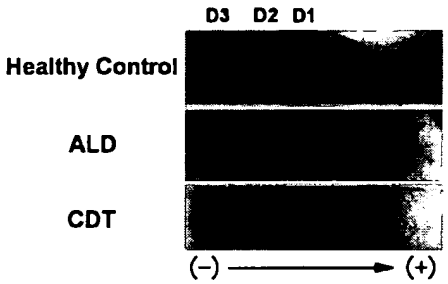

Figure 3. Patterns of desialylated transferrin bands separated by DSA-affinity electrophoresis.

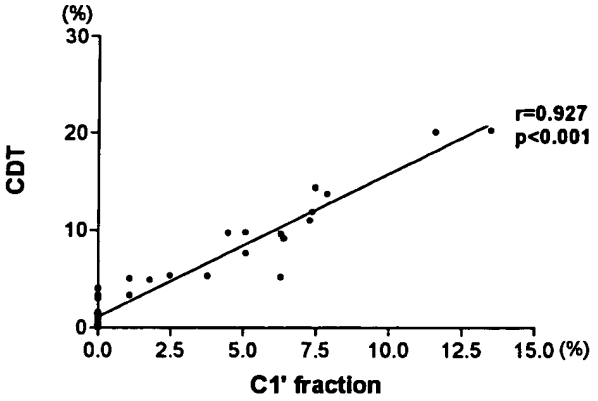

Figure 4. Correlation between the serum levels of CDT and the proportions of C1′.

desialylated transferrin from healthy controls and ALD patients were separated into three bands – a nonreactive main band (D1), a weakly reactive band (D2), and a strongly DSA-reactive band (D3). Serum CDT partially purified from ALD patients consisted of two bands with a trace amount of D3 and a major D1 whose electrophoretic mobility corresponded to that of F2 in lectin-free agarose gel electrophoresis (data not shown). D1 obtained from ALD patients was slightly broader than that from healthy controls because of probable contamination by fast migrating transferrin molecules as detected in serum CDT. A significant correlation was found between the proportion of C1′ in the sera from ALD patients and the serum levels of CDT ($r = 0.927$, $P < 0.001$; Fig. 4). C2′ in the sera from ALD patients could not be quantitated by densitometry because of incomplete separation of C2′ from C3. The proportion of C1′ in the sera from ALD patients tended to be correlated with serum γ-glutamyl transpeptidase activity, but not with asparate aminotransferase activity, alanine aminotransferase activity, nor the erythrocyte mean corpuscular volume, respectively (data not shown). In the sera from ALD patients, C1′ appeared when the serum CDT level was above 4%, which was statistically significant in ALD patients using the Axis CDT kit [20].

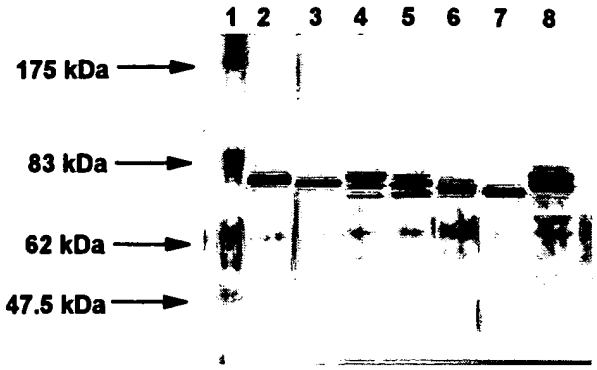

Figure 5. SDS-PAGE of serum transferrin followed by Western blotting analysis. Lane (1) molecular markers (BioLabs), MBP-b-galactosidase (175 kDa), MBP-paramyosin (83 kDa), glutamic dehydrogenase (62 kDa), and aldolase (47.5 kDa); (2) serum transferrin from a healthy control; (3) desialylated control transferrin; (4) serum transferrin from an ALD patient; (5) serum CDT; (6) desialylated CDT; (7) serum transferrin with complete digestion by *N*-glycosidase F; (8) serum transferrin with incomplete digestion by *N*-glycosidase F.

3.2 Western blot analysis

Serum transferrin from healthy controls yielded a single band of 78 kDa (Fig. 5, lane 2), while serum transferrin from ALD patients consisted of a main band of 78 kDa and two additional bands estimated to be approximately 75 and 72 kDa (lane 4). The molecular masses of these additional bands corresponded to those of two major bands of serum CDT partially purified from ALD patients (lane 5) and were smaller than that of desialylated control transferrin (lane 3). Neuraminidase treatment of serum CDT diminished the molecular mass of the 75 kDa band, but did not affect the 72 kDa band (lane 6). After complete digestion by *N*-glycosidase F, the molecular mass of the control transferrin was decreased. Completely deglycosylated transferrin molecules estimated to be 72 kDa were compatible with the smallest molecular mass of serum CDT separated by SDS-PAGE (lane 7). By contrast, incomplete digestion of transferrin by *N*-glycosidase F revealed two bands estimated to be 78 and 75 kDa, respectively. The 78 kDa band corresponded to an indigestible intact transferrin and the 75 kDa band was compatible with the middle band of serum CDT (lane 8). After four weeks of alcohol abstinence, the serum CDT bands were scarcely detectable (data not shown).

4 Discussion

By lectin affinity electrophoresis and SDS-PAGE analysis, we demonstrated that serum CDT consisted of transferrin isoforms lacking one or both of the entire carbohydrate

chains. Con A-affinity electrophoresis separated serum transferrin from ALD patients into five bands, of which two additional bands (C1′ and C2′) constituted the predominant components of serum CDT. SDS-PAGE followed by Western blot analysis also revealed that serum transferrin from ALD patients consisted of a major 78 kDa band and two additional bands (75 and 72 kDa) constituting the predominant components of serum CDT. Since a glycan chain with *N*-linked complex type oligosaccharides accounts for about 2.4–4 kDa depending on whether the chain is bi-, tri-, or tetra-antennary, serum CDT with an approximately 3 and 6 kDa smaller molecular mass than normal transferrin might be comparable to monoglycosylated and aglycosylated transferrin, respectively. Several investigations were carried out in order to clarify the molecular characteristics of C1′ and C2′ of serum CDT.

When serum CDT was subjected to electrophoresis in agarose gel with DSA or without lectin, the electrophoretic mobility of the fast migrating band in D1 obtained from DSA-containing gel electrophoresis as well as that of F2 from lectin-free agarose gel electrophoresis was similar in value to that of C1′ obtained from Con A-containing gel electrophoresis. Furthermore, desialylation reaction did not affect the electrophoretic mobility of C1′. It is well-known that Con A has an affinity for the trimannosyl core in biantennary complex-type oligosaccharides and DSA binds strongly to tri- and tetraantennary complex-type oligosaccharides with C-2,6 outer chain branching [8, 9, 16, 17]. DSA also binds weakly to C-2,4-branched trianntenary oligosaccharides [16, 17]. These data indicated that the electrophoretic mobility of C1′ is based only on the p*I* value of the transferrin molecule, but not on lectin affinity. Because of the lack of lectin affinity as well as neuraminidase sensitivity, C1′ was considered to represent a transferrin isoform with either complete deglycosylation or two incomplete oligosaccharide chains without a trimannosyl core. This assumption was supported by two results from comparative SDS-PAGE followed by Western blot analysis of serum CDT and serum transferrin from the healthy controls: (i) Complete *N*-glycosidase F treatment with control transferrin gave rise to a single band of 72 kDa, which was the same molecular mass as the smallest band of serum CDT. (ii) The smallest (72 kDa) band in serum CDT showed no change in molecular mass after neuraminidase treatment. Consequently, these results suggested that transferrin molecules of C1′ were completely deficient in asparagine-*N*-linked oligosaccharide. By contrast, C2′ showed weak, but obvious, affinity for Con A because of increased cathodic migration as compared to C1′, which showed the same mobility (as F2) in lectin-free agarose gel electrophoresis. Moreover, the electrophoretic mobility of C2′ was reduced significantly by treatment with neuraminidase. In SDS-PAGE gel, the reduced molecular mass of the middle band (75 kDa) in serum CDT was noted after neuraminidase treatment. Incomplete digestion of control transferrin with *N*-glycosidase F generated a 75 kDa band mixed with an undigested 78 kDa band. This result suggested that C2′ probably consisted of transferrin with one *N*-linked complex-type oligosaccharide. In order to confirm our result, several serum samples digested with *N*-glycosidase F were further analyzed by Con A-affinity electrophoresis. However, resultant digested transferrin could not be detected by the antibody-affinity blotting, because the presence of 2-mercaptoethanol and/or *N*-glycosidase F might have inhibited the reaction.

Our present study detected slight serum CDT with a strong Con A-reactive nature and with a molecular mass of about 78 kDa. This can be attributed to the fact that the columns of the Axis %CDTri TIA kit can collect not only asialo, monosialo and disialo transferrin without a single trimannosyl core, but also trisialo transferrin with two trimannosyl cores as described in the manufacturer's instructions. The results of the analyses combined with lectin affinity electrophoresis and SDS-PAGE suggested that the C1′ and C2′ detectable in ALD serum consisted of biantennary monoglycosylated and aglycosylated transferrin, respectively. The present study concluded that serum CDT might lack at least one glycan chain, but not an antenna of the glycan chain. It has been reported that the components of serum transferrin with high p*I* values, and whose charge corresponds to disialo- (p*I* 5.7) as well as, to a lesser degree, mono- and asialotransferrin (p*I* 5.8 and 5.9, respectively), are present in the sera from ALD patients [1–5]. Our present results indicate the possibility that the greater part of serum transferrin with p*I* 5.7 is biantennary-, monoglycosylated disialotransferrin derived from C2′, and the majority of the p*I* 5.9 transferrin is aglycosylated asialotransferrin from C1′. The same conclusion has been reported using high-pH anion-exchange chromatography [11]. The present study also confirms that serum CDT consisted of transferrin isoforms lacking one or both of the entire carbohydrate chains using lectin affinity electrophoresis and SDS-PAGE analysis. Though our previous study did not detect C1′ and C2′, our present study obtained good resolution of the additional bands as a result of prolonging the electrophoretic distance and changing the dilution of the samples. To detect transferrin isoforms that do not lack one or both of the entire carbohydrate chains in ALD patients, lectin affinity electrophoresis coupled with the antibody affinity blotting method is more practical for clinical use than chromatographic analyses.

The mechanism by which alcohol consumption causes elevation of serum CDT levels is still not understood.

Several reports have provided evidence that alcohol abuse may inhibit glycosylation of glycoprotein in the Golgi apparatus, involving reduced activities of glycoprotein glycosyltransferase and enhanced activities of lysosomal β-hexosaminidase and neuraminidase [21–26]. Since our studies suggested that one or two potential oligosaccharide sites of serum CDT from ALD patients were aglycosylated, synthesis of serum CDT molecules might be due not to unusual post-translational processing, but to temporal suppression of the biosynthetic enzyme(s) for dolichol-oligosaccharide intermediates or an *N*-linked oligosaccharide transferase. Moreover, the production of serum CDT molecules may be due to abnormal activation of "unknown" glycosidase(s).

In conclusion, our findings confirmed that sugar chains of partially purified serum CDT obtained from patients with ALD exhibit not merely a loss of terminal sialic acid, but also the absence of the *N*-linked complex type oligosaccharide chain itself. Furthermore, this method can avoid the complicated manipulation of serum samples and may serve as a useful tool for the detection and monitoring of ALD. The biological similarity between the acquired transferrin change in ALD and that in type I carbohydrate-deficient glycoprotein syndrome should be investigated to provide clues to the metabolic background of the effects of alcohol abuse on glycoprotein metabolism [27].

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Expression of glycosylated and nonglycosylated human transferrin in mammalian cells. Characterization of the recombinant proteins with comparison to three commercially available transferrins.

Mason AB, Miller MK, Funk WD, Banfield DK, Savage KJ, Oliver RW, Green BN, MacGillivray RT, Woodworth RC.

Department of Biochemistry, University of Vermont College of Medicine, Burlington 05405.

The coding sequence for human serum transferrin was assembled from restriction fragments derived from a full-length cDNA clone isolated from a human liver cDNA library. The assembled clone was inserted into the expression vector pNUT and stably transfected into transformed baby hamster kidney (BHK) cells, leading to secretion of up to 125 mg/L recombinant protein into the tissue culture medium. As judged by mobility on NaDodSO4-PAGE, immunoreactivity, spectral properties (indicative of correct folding and iron binding), and the ability to bind to receptors on a human cell line, initial studies showed that the recombinant transferrin, is identical to three commercial human serum transferrin samples. Electrospray mass spectrometry (ESMS), anion-exchange chromatography, and urea gel analysis showed that the recombinant protein has an extremely complex carbohydrate pattern with 16 separate masses ranging from 78,833 to 80,802 daltons. Mutation of the two asparagine carbohydrate linkage sites to aspartic acid residues led to the expression and secretion of up to 25 mg/L nonglycosylated transferrin. ESMS, anion-exchange chromatography, and urea gel analysis showed a single molecular species that was consistent with the expected theoretical mass of 75,143 daltons. In equilibrium binding experiments, the nonglycosylated mutant bound to HeLa S3 cells with the same avidity and to the same extent as the glycosylated protein and the three commercial samples. These studies demonstrate conclusively that carbohydrate has no role in this function.

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